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Review

Review of Shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy production

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ABSTRACT

The involvement of the pathogenic Shiga-toxin-producing *Escherichia coli* (STEC; also called verocytotoxic-producing *E. coli* or VTEC) in sporadic cases and disease outbreaks is presently increasing. Infrequent cases are due to ingestion of milk and dairy products. As ruminants are healthy carriers of STEC and most dairy products may provide these bacteria with favourable conditions for their growth, milk and dairy products are a potential source of STEC. But not all STEC serotypes are pathogens; only relatively small numbers in the entire family of STEC are pathogenic. This review focuses on the recent advances in understanding of STEC and their significance in milk and dairy products. It is intended to gather the information that is needed to understand how these bacteria are described, detected and characterised, how they contaminate milk and grow in dairy products, and how the dairy industry can prevent them from affecting the consumer.

Contents

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1. Introduction

1.1. Background

According to the Food and Agriculture Organization of the United Nations, world milk production reached 724 million tonnes in 2010, resulting in an enormous consumption of and trade in dairy products (FAO, 2011). In this regard, safety and the control/prevention of milk-borne food pathogens are of primary importance to public health. Farmed animals represent a major reservoir of pathogens that can be transferred to milk; the predominant human bacterial pathogens that can potentially be transferred to milk include *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter* spp. and pathogenic *Escherichia coli*. Raw milk provides a potential growth medium for these bacteria.

E. coli is a Gram-negative, facultative anaerobe, within the family Enterobacteriaceae, and is normally a commensal bacterium that co-exists with its human host in the intestines in a mutually beneficial relationship (Tchaptchet and Hansen, 2011). For the most part, *E. coli* is a group of harmless bacteria that are most often used as indicator organisms for faecal contamination and breaches in hygiene. However, several *E. coli* clones have acquired virulence factors that have allowed them to adapt to new niches and in some cases to cause serious disease. There are six categories of pathogenic *E. coli* that affect the intestines of humans: Shiga-toxin-producing *E. coli* (STEC; also called verocytotoxin-producing *E. coli* or VTEC), of which enterohaemorrhagic *E. coli* (EHEC) are a pathogenic sub-group; enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroaggregative *E. coli* (EAEC); enteroinvasive *E. coli* (EIEC); and diffusely adherent *E. coli* (DAEC).

Among the pathogenic *E. coli* of greatest relevance to milk is *E. coli* O157:H7, an STEC serotype, which, because of its high virulence (it can cause disease at a dose of 5–50 cells), is of major concern to the dairy industry. According to a recent review (Mathusa et al., 2010), six other non-O157 STEC serogroups are emerging as leading causes of infection: *E. coli* O26, O103, O111, O121, O45 and O145 (in order of frequency in the US). According to a European Food Safety Authority report (EFSA, 2009a), the major serotypes or serogroups of concern are *E. coli* O157:H7, O26, O103, O145, O111, and O91. These serogroups are a small number in the entire family of STEC. Water and foodstuffs that are subject to faecal contamination from ruminants could represent a threat to food safety. In North America, Japan and parts of Europe, most outbreaks have been caused by strains belonging to the serotype O157:H7; however, other serogroups are also an important health concern.

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1.2. Purpose of this review

The purpose of this review is to assess the current situation with respect to the major serotypes of pathogenic STEC in dairy products. Twelve years ago, the International Dairy Federation (IDF) established a Task Force comprising scientists involved in research on pathogenic *E. coli* to review and assess the significance of STEC in dairy production with respect to consumer health (Bastian and Sivelä, 2000).

As cheesemaking is a major sector of the dairy industry worldwide, and many cheese varieties throughout the world are typically made from raw or unpasteurised milk with natural enzymes and microflora that are responsible for enhancing desirable flavour characteristics (Fox and McSweeney, 2004), this review has a focus on cheese with respect to pathogenic STEC serogroups.

The review first describes the bacteriological characteristics of STEC, its sources and its mode of transmission. This is followed by a description of the analytical methods, including testing for virulence factors, to detect and identify the human pathogenic STEC strains/serotypes in milk and dairy products. Then the main outbreaks associated with dairy products, the occurrence of STEC in raw milk and cheeses and its survival during cheesemaking are discussed. Finally, measures that can help prevent the contamination of milk and dairy products are proposed. The document focuses on recent advances in the understanding of the strains and/or serotypes that pose a significant risk to human health.

2. Bacteriological characteristics

2.1. Shiga-toxin-producing E. coli

E. coli is a micro-organism that is commonly found in the lower intestinal tract of healthy humans and animals. There are many types of *E. coli*, a few of which are potentially pathogenic by a variety of infective and toxin-producing mechanisms. Symptoms vary according to the strain of *E. coli* encountered and the resistance of the individual to such illness. Infants, young children, the elderly and the immunocompromised are generally more susceptible to *E. coli* infections than healthy older children and adults, although the recent *E. coli* O104 outbreak in Germany caused more problems in healthy adults (EFSA, 2011a), indicating that such generalisations do have significant variations. All STECs have the same morphology. They are Gram-negative bacilli belonging to the Enterobacteriaceae family and the *Escherichia* genus. STECs are characterised by their serogroup, virulence genes, toxins and associated disease symptoms. STECs are so named because they produce one or more cytotoxins, called Shiga toxin 1 (stx1) and Shiga toxin 2 (stx2).

2.2. Growth and inactivation

Most *E. coli* grow between 10 and 46 °C, with some strains growing at 8 °C (ICMSF, 1996). However, some STEC serotypes can grow in milk at temperatures as low as 6.5 °C (Kauppi et al., 1996). A recent publication has also shown that *E. coli* O157 strains possess inherent genetic mechanisms which enable growth at low temperatures (<15 °C), compared to non-pathogenic *E. coli* (Vidovic et al., 2011). STECs, like other Gram-negative bacteria, do not exhibit unusual heat resistance under conditions of neutral pH and moderately high water activity (a_w; e.g. 0.95; Kaur et al., 1998). Pasteurisation of milk at 72 °C for 15 s inactivates O157:H7 (D'Aoust et al., 1988).

2.3. Resistance to environmental stress

In bacteria, stress resistance mechanisms are a common survival strategy. This is particularly relevant to bacterial survival in food. Most survival studies (in food or laboratory media) have been undertaken using *E. coli* O157:H7 strains and little is known about other STEC serotypes.

STEC O157:H7 have been shown to survive at temperatures that are lower than those suitable for growth. For example, *E. coli* O157:H7 can survive for several weeks or months in meat, fruits, ice cream and yoghurt when frozen at −18 to −20 °C (De Schrijver et al., 2008; Doyle and Schoeni, 1984; Grzadkowska and Griffiths, 2001; Strawn and Danyluk, 2010), and outbreaks have been linked to frozen ground beef patties that contained viable *E. coli* O157:H7 (CDC, 1997, 2000).

Environmental stresses can induce thermotolerance in some strains. Exposure to mild heat, or cross-protection induced during acid adaptation, has been reported to increase thermotolerance by up-regulating stress response genes (Murano and Pierson, 1992; Ryu and Beuchat, 1998, 1999; Semanchek and Golden, 1998). Fat also has a protective effect against thermal inactivation (Line et al., 1991; Singh and Ranganathan, 1980). Three mechanisms in *E. coli* allow the cells to withstand acid challenge at pH 2.5: the RpoS, the arginine decarboxylase and the glutamate decarboxylase systems (Castanie-Cornet et al., 1999). Acid resistance of STEC is mediated by *rpoS*, which encodes a stationary-phase sigma factor. This feature helps STEC to survive the acidity of the stomach and to colonise the gastrointestinal tract. Furthermore, it also increases the survival of STEC in acidic foods, enabling survival for extended periods, particularly at refrigeration temperature (Meng et al., 2007; Semanchek and Golden, 1996). Hence, contaminated cultured and fermented foods such as yoghurt and cheese have been implicated in sporadic cases and outbreaks (Baylis, 2009; Besser et al., 1993; CDC, 1995a, 2000; Cody et al., 1999; Ethelberg et al., 2007, 2009; Hussein and Bollinger, 2005; Hussein and Sakuma, 2005a; King et al., 2010; Sartz et al., 2008; Weagant et al., 1994).

Although there are relatively few published studies evaluating the survival of non-O157 STEC in foods, isolating non-O157 from commercial product suggests that these strains may be equally resistant to stress conditions (Baylis, 2009; Stephan et al., 2008).

Other preservatives have various effects on STEC. Lactate, propionate, sorbate and benzoate inhibit non-O157 STEC but the extent of inhibition or survival is concentration and temperature dependent (Hazarika et al., 2003; McWilliam Leitch and Stewart, 2002a, 2002b).

3. Detection and isolation methods

STECs comprise over 400 serotypes that differ greatly in both their physiological characteristics and their pathogenic potential to humans (Bettelheim, 2007; Scheutz and Strockbine, 2005); at present it is still not possible to fully define human pathogenic STECs (AFSSA, 2010; EFSA, 2007). This diversity is reflected in the use of very different detection methods for STEC in monitoring programmes or official food controls and in the lack of international standardised procedures for detection of non-O157 STEC. Generally, two different approaches are used to detect STEC in foods: 1) serogroup-independent techniques where data on the prevalence of genes and the characteristics of STEC occurring in food is acquired (Beutin et al., 2007; Fach et al., 2001). This can result in isolation of non-disease causing strains. 2) Serogroup-dependent methods which target a subset of serogroups most frequently involved in human disease and outbreaks and focus on STECs likely to exhibit virulence in humans, such as strains belonging to serogroups O26, O45, O91, O103, O111, O121, O145 and O157 (Auvray et al., 2007; EFSA, 2009a; Fratamico et al., 2011; Madic et al., 2009, 2011; Perelle et al., 2007). Food samples contaminated by STEC serotypes that are less frequently associated with human infections, but can still cause cases of HUS (e.g. O113:H21, O174:H21, or the newly emerging O104:H4 serotype; [Scheutz et al., 2011]) are missed by these selective methods.

As *E. coli* O157:H7 is the most prevalent cause of STEC human disease and outbreaks, genetic detection assays and differential/selective agars for its isolation are well developed and widely available for routine analysis. Non-O157 STEC serogroups have also been increasingly linked to food-borne illness outbreaks and severe pathology (see Section 7), and therefore genetic detection methods and differential/selective agars for isolating the most prevalent non-O157 serogroups (i.e. O26, O103,

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O111 and O145) have recently been developed. However, as described below, additional method development for these and other STEC serogroups is still required.

3.1. Culture and isolation methods

3.1.1. Enrichment

As food samples usually contain low numbers of STEC together with a high level of competing microflora, a selective enrichment step is required for the isolation of STEC. For processed foods, enrichment may also facilitate the recovery of injured or stressed bacterial cells that have been exposed to harsh manufacturing processes. A wide range of enrichment protocols, which use different basal broths, different added selective agents and different incubation times and temperatures, have been described (Vimont et al., 2006). Although various enrichment protocols have been compared for their ability to support growth of STEC, no clear conclusions could be drawn regarding their relative efficacy (Baylis, 2008; Vimont et al., 2006); to date, there are no standard enrichment conditions for STEC serotypes other than O157:H7. Studies have suggested that all serotypes cannot be detected by one method (Baylis, 2008).

For the detection of *E. coli* O157:H7 and non-O157 STEC, tryptone soya broth (TSB) and *E. coli* broth (EC), incubated at 35–37 °C for 16–24 h, are the most frequently employed enrichment media (Vimont et al., 2006). To suppress the antagonistic activity of the background microflora, incubation at 42 °C may be used although this temperature may also interfere with the recovery of injured STEC cells. Enrichment broths are frequently supplemented with selective agents such as potassium tellurite and antibiotics, especially novobiocin (or acriflavin in the case of dairy products). However, it has been reported that systematic addition of novobiocin should be avoided as it may inhibit the growth of some STEC strains (Vimont et al., 2006). Jasson et al. (2009) also showed a susceptibility of STEC O157 to various selective components in the enrichment medium and therefore supported the use of non-selective media, such as buffered peptone water (BPW).

3.1.2. Immunomagnetic separation

By taking advantage of interactions between antibodies and O-antigens, immunomagnetic separation (IMS) can be used, after enrichment and prior to plating, for the selective concentration of STEC cells belonging to particular serogroups. The use of IMS-O157 is well established and is considered to be the official standard method for the detection of *E. coli* O157 in foods and animal feedstuffs (ISO, 2001). It was shown to be effective and applicable to the examination of dairy products, with a detection limit of 1–2 CFU/25 g (Voitoux et al., 2002). Automated IMS technology allowing sample recirculation over a "capture phase" has also been proposed to improve STEC recovery (Fedio et al., 2011).

IMS-based methods have not yet been standardised for the detection of non-O157 STEC. Commercial IMS assays are available for a subset of four non-O157 serogroups most frequently involved in HUS and outbreaks, i.e. O26, O103, O111 and O145. However, the detection of these non-O157 STEC by IMS is subject to serotype-specific variations in capture efficiency (Auvray et al., 2007; Hara-Kudo et al., 2000; Jenkins et al., 2003; Madic et al., 2011; Verstraete et al., 2010).

3.1.3. Selective media

In contrast to commensal *E. coli* strains, typical *E. coli* O157:H7 do not ferment sorbitol, lack the ability to produce β-D-glucuronidase (GUD) and are also more resistant to cefixime and tellurite. Based on one or more of these properties, several solid growth media, including sorbitol-MacConkey (SMAC) agar and its cefixime-tellurite-supplemented formula (CT-SMAC), have been developed (March and Ratnam, 1986; Zadik et al., 1993). Alternative agars, mostly based on chromogenic reactions, distinguishing *E. coli* O157:H7 from background flora, are commercially available. However, these media not only fail to detect all the non-O157 STEC serotypes, but also fail to detect atypical (HUS-causing) *E. coli* O157:H7/H$^-$ strains, which are sorbitol fermenting (SF) and GUD-positive (and therefore called sorbitol-positive/SF O157 [Karch and Bielaszewska, 2001]). In addition, several non-O157 *E. coli* serogroups lack sorbitol fermentation and GUD activity (Manna et al., 2010), which complicates phenotypic identification of *E. coli* O157.

The enterohaemolytic phenotype of a significant proportion of STEC strains has been exploited for the development of an 'enterohaemolysin agar' prepared with washed sheep erythrocytes supplemented with Ca^{2+} and vancomycin–cefsulodin–cefixime for increased selectivity. After incubation at 37 °C for 18–24 h, enterohaemolytic colonies are identified by the formation of small turbid haemolytic zones (Beutin et al., 1989). However, it should be stressed that non-haemolytic STEC will be missed on this medium.

As most strains of STEC O26 are unable to ferment rhamnose and, like O157:H7, are also more resistant to cefixime and tellurite, rhamnose-MacConkey (RMAC) agar and RMAC agar supplemented with cefixime and tellurite (CT-RMAC) are often used to differentiate STEC O26 from other *E. coli* (Hiramatsu et al., 2002). Recently, new selective differential and confirmation media, based on a mixture of carbohydrate sources and a chromogenic substrate for β-D-galactosidase, have been developed and evaluated for isolating *E. coli* O26, O103, O111 and O145, as well as sorbitol-positive and -negative O157 STEC at levels of ≤100 CFU/25 g (Possé et al., 2008a, 2008b). Overall isolation efficiencies ranged from 100% for raw milk, to 82.3% and 88.5% for cheeses made with pasteurised milk and raw milk, respectively (Possé et al., 2008b). Cold-stressed and non-stressed STEC O26, O103, O111 and O145 inoculated into the cheese samples at levels of 10–30 CFU/25 g were reliably isolated using this procedure. Plating on Rainbow® Agar O157 was also successfully used for isolation of *E. coli* O26, O45, O103, O111, O121 and O145 from artificially contaminated ground beef (Fratamico et al., 2011). Finally, CHROMagar™ STEC (CHROMagar Microbiology) was found suitable for the selective screening of major EHEC serotypes (e.g. O26:H11, O111:H8, O118: H16, O121: H19, O145:H28 and O157:H7) from vegetables (Tzschoppe et al., 2012).

3.1.4. Colony hybridisation and immunoblot assays

Despite the lack of isolation media for the majority of STEC serogroups, suspect *stx*-positive colonies isolated on agar plates can be identified using the DNA probe hybridisation technique (Karch and Meyer, 1989). For the detection of STEC in dairy products, diluted aliquots of enriched cultures, displaying a positive polymerase chain reaction (PCR) result for *stx*, can be plated by spreading onto agar before applying the filter technique for blotting and hybridisation (Fach et al., 2001; Messelhäusser et al., 2008; Parisi et al., 2010; Stephan et al., 2008; Vernozy-Rozand et al., 2005a). Alternatively, identification of STEC isolates can be performed immunologically using anti-Stx antibodies. However, colony hybridisation or immunoblot assays are rather laborious techniques that cannot be easily performed routinely. Moreover, the detection of low numbers of STEC is difficult, as STEC may be outgrown by the background microflora on the agar plates.

3.1.5. Confirmation of suspected colonies

Isolates of STEC have to be confirmed on two levels: the serotype and the production of Stx or the presence of *stx* genes. Several different approaches are used, including serological techniques, tissue culture assay and nucleic acid-based methods.

Traditionally, suspected colonies on selective media are confirmed biochemically as *E. coli* and serotyped. However, large sets of antisera against *E. coli* O-antigens 1–181 and H-antigens 1–56 are restricted to specialised international reference laboratories and only a limited number of O- and H-antigens can be typed using commercial serological kits. The production of active Stx can be tested biologically using the vero cell assay (VCA) (Konowalchuk et al., 1977). Although this assay is considered to be the gold standard, its use in routine diagnostic laboratories is limited because it is time consuming and

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requires facilities for cell culture preparation. Commercial immunoassays and reverse passive latex agglutination (RPLA) tests are also available for Stx detection (see: http://denka-seiken.jp/english/products/bacteriology/escherichiaColi.html).

Alternatively serotyping and examination of virulence can be achieved using DNA-based molecular diagnostic tools. A large number of conventional PCR tests targeting *stx*, *eae* and genetic markers specific for the O- and H-antigens associated with the main pathogenic STEC serotypes have been developed, including simplex and multiplex assays (Bastian et al., 1998; Botteldoorn et al., 2003; Monday et al., 2007; Paton and Paton, 1998). Real-time PCR methods based on the use of fluorescent dyes or fluorogenic oligonucleotide probes have been proposed, offering interesting advantages, such as quantification, increased speed, prevention of carryover contamination and high throughput automated analysis (Beutin et al., 2009; Bugarel et al., 2010a, 2010b; Fratamico et al., 2009, 2011; Lin et al., 2011; Madic et al., 2010; Nielsen and Andersen, 2003; O'Hanlon et al., 2004; Perelle et al., 2004). DNA microarray technology has also been used for rapid genetic identification of 24 O groups and 47 H types, representing a valuable alternative to classical serotyping (Ballmer et al., 2007).

3.2. Methods for the detection of STEC in food

3.2.1. Culture-based methods

An International Organization for Standardization (ISO) method is available for the detection of *E. coli* O157:H7 in food and animal feedstuffs (ISO, 2001). This method is based on an enrichment procedure, followed by a separation and concentration step, and then an isolation step on selective chromogenic media. It was shown to be sensitive, with a detection limit of 1–2 CFU/25 g, as well as effective and applicable to the examination of dairy products (Voitoux et al., 2002). Methods for standardisation of non-O157 STEC are in the process of being developed.

One of the disadvantages with culture-based methods is that cells can enter a dormancy state where they become non-culturable (viable but non-culturable [VBNC]; Dinu and Bach, 2011). This can lead to an underestimation of numbers or a failure to isolate a viable culture, although the cells may still retain pathogenicity, or be recoverable to a viable cell state. Recovery of cells may be compromised by selective or chromogenic media and VBNC should be considered in using these media.

3.2.2. Molecular-based and immunological methods

Recently, a real-time PCR-based method for detection of pathogenic STEC belonging to serogroups O26, O103, O111, O145 and O157 has been developed by Working Group 6 of Technical Committee 275 of CEN (European Committee for Standardisation) and has since been proposed as a draft International Organization for Standardization Technical Specification (ISO/TS 13136; EFSA, 2009a). It is intended for the analysis of human food and animal feedstuffs, as well as for environmental samples in the area of food production and/or handling and at primary production stages.

Real-time PCR is recognised as a highly specific and sensitive technique that can be completed in 2–4 h after enrichment. Its potential for automation also makes it suitable for the screening of large numbers of food samples. Table 1 includes a list of commercialised real-time PCR tests allowing the detection of *E. coli* O157 or *stx* genes in foods, with analytical performances, approved by AFNOR Validation or AOAC International. Numerous other PCR methods targeting STEC-associated markers have been described in the literature and could be useful for screening food. However, only a few of these include an IAC to detect PCR failures caused by inhibitory food compounds (Auvray et al., 2009; Belanger et al., 2002; Derzelle et al., 2011; Fratamico et al., 2009, 2011; Stefan et al., 2007). In addition, failure of some PCR assays to detect genetically distant *stx* variants has been described (Bastian et al., 1998; Beutin et al., 2009; Reischl et al., 2002). Recently, a quantitative PCR assay designed to detect all known *stx* gene subtypes including the most distant variant *stx*2f was developed and successfully applied for STEC detection in foods such as raw milk cheeses (Derzelle et al., 2011). This assay also proved to be appropriate for the direct quantification of STEC in milk over a 5 log dynamic range, i.e. from 4×10^6 to 40 CFU/mL.

Table 1
Validated commercial immunoassays and PCR tests for the detection of *E. coli* O157 or STEC in foods (as at August 23rd 2011).

Principle[a]	Name of the assay (manufacturer)	Validation
AIEA	VIDAS® *E. coli* O157 (ECO), VIDAS® UP *E. coli* O157 (including H7) (bioMérieux)	AOAC/AFNOR
ELISA	Assurance EIA® EHEC (BioControl Systems)	AOAC
	TECRA® *E. coli* O157 VIA™ (3 M TECRA)	AOAC
LFI	DuPont™ Lateral Flow System *E. coli* O157 (DuPont Qualicon)	AOAC
	RapidChek® Select™ *E. coli* O157 (Strategic Diagnostics)	AOAC
	Reveal® 2.0 *E. coli* O157:H7 test system (Neogen Corporation)	AOAC
	Singlepath® *E. coli* O157 (Merck/EMD Chemicals)	AOAC
	VIP Gold ™ for EHEC (BioControl Systems)	AOAC
MICT	FoodChek™ *E. coli* O157 (FoodChek Systems)	AOAC
Real-time PCR	ADIAFOOD Rapid Pathogen Detection system for *E. coli* O157, ADIAFOOD Rapid Pathogen Detection system for *E. coli* O157:H7 (AES Chemunex)	AOAC
	Assurance GDS™ Shiga Toxin Genes, Assurance GDS™ *E. coli* O157:H7 (BioControl Systems)	AOAC
	BAX® *E. coli* O157:H7 MP, BAX® Real-time PCR assay *E. coli* O157:H7 (DuPont Qualicon)	AOAC/AFNOR
	E. coli O157:H7 LT Test Kit (Idaho Technology)	AOAC
	Foodproof® *E. coli* O157 Detection Kit (BIOTECON Diagnostics GmbH)	AOAC
	GeneDisc pathogenic *E. coli* O157, GeneDisc STEC, GeneDisc EHEC 5 ID (Pall GeneDisc Technologies)	AOAC/AFNOR
	HQS *E. coli* O157:H7 (ADNucleis)	AFNOR
	IEH *E. coli* O157, Stx-producing *E. coli* (STEC) with Intimin (IEH Laboratories and Consulting Group)	AOAC
	iQ-Check™ *E. coli* O157:H7 (Bio-Rad)	AOAC/AFNOR
	MicroSEQ® *E. coli* O157:H7 Detection kit (Applied Biosystems)	AOAC/AFNOR

[a] AIEA, automated immunoenzymatic assay; ELISA, enzyme-linked immunosorbent assay; LFI, lateral flow immunoassay; MICT, magnetic immunochromatographic test.

Multiparametric real-time PCR-based approaches targeting several EHEC virulence genetic markers facilitate the identification of food samples contaminated by STECs belonging to various serogroups such as O26, O103, O111, O145 and O157 (Auvray et al., 2007; Perelle et al., 2007) or O26, O45, O103, O111, O121, and O145 (Fratamico et al., 2011). The interest of a sequential molecular strategy for the detection of STEC O26:H11, O103:H2, O111:H8, O145:H28 and O157:H7 in cheeses was also described recently (Madic et al., 2010, 2011). By targeting associations of genetic markers specific for highly pathogenic STEC, such methods help to further refine the diagnostic results obtained from PCR-based analysis of complex food matrices (Fig. 1).

In addition to PCR-based methods, many immunoassays easy to perform in routine laboratories have been developed and validated for the screening of *E. coli* O157 in foods (Table 1). Recently, a lateral flow device capable of detecting STEC serogroups O26, O45, O103, O111, O121 and O145 inoculated at concentrations of 3–6 CFU/375 g of beef after 18 h enrichment at 42 °C has been reported (Nadala and Samadpour, 2011). Further developments of this nature can be expected in the coming years.

In contrast to cultural methods which are time-consuming, labour-intensive, and often associated with a low rate of STEC recovery from food, PCR- or immunoassay-based methods are characterised by reduced analysis time and high-throughput analysis capabilities. In particular, they offer the possibility to quickly rule out negative samples, which represent the majority of food samples (Lynch et al., 2012; Perelle et al., 2007; Thomas et al., 2011). Only samples that test positive require confirmation by cultural methods.

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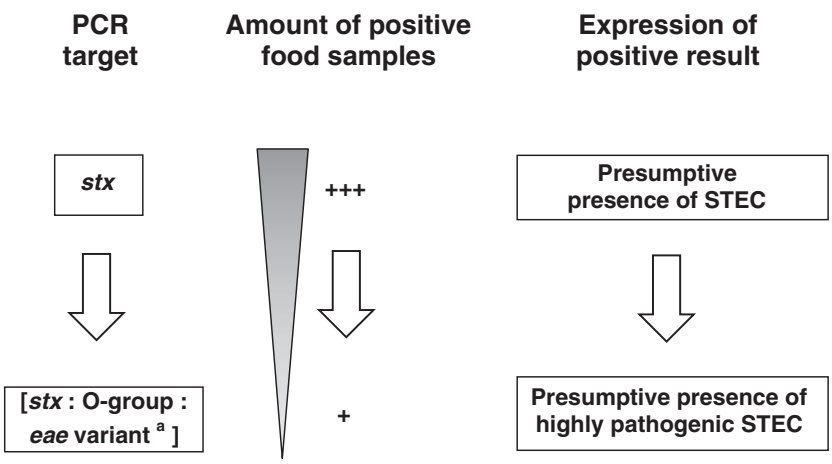

Fig. 1. Schematic presentation of the interest of real-time PCR-based detection of genetic allelic combinations from the main pathogenic STEC for food analysis. [a]Combinations of genetic markers typical of STEC O26:H11, O103:H2, O111:H8, O145:H28 and O157:H7 are *stx*:wzx_{O26}:*eae-β1*, *stx*:wzx_{O103}:*eae-ε*, *stx*:$wbdl_{O111}$:*eae-γ2/θ*, *stx*:$ihp1_{O145}$:*eae-γ1* and *stx*:$rfbE_{O157}$:*eae-γ1*, respectively.

A limitation of these rapid screening methods however is that they are restricted to a small number of pathogenic STEC serogroups. Although adaptation of these techniques can be achieved easily for the detection of emerging serotypes such as O104:H4 (Scheutz et al., 2011; Tzschoppe et al., 2012), potentially virulent STEC strains belonging to serotypes less frequently involved in human disease would still remain undetected during routine food analysis. Screening methods targeting virulence gene profiles of EHEC irrespective of their serotypes would therefore seem better suited. Unfortunately, various virulence gene profiles occur among pathogenic STEC strains and the full combinations of virulence factors necessary to cause disease still remain to be defined, which hampers the development of such serotype-independent screening strategies.

3.3. Genetic typing of STEC isolates

Further genetic characterisation of STEC isolates can facilitate epidemiological investigation of outbreaks. Because of its high level of discrimination, pulsed-field gel electrophoresis (PFGE) is considered to be the standard method for the typing of STEC, especially if use of the standardised PulseNet protocol (http://www.pulsenetinternational.org) is made. Subtyping of STEC virulence genes using PCR-based techniques, microarrays or DNA sequencing has also proven to be useful for epidemiological and phylogenetic analyses (Nielsen and Andersen, 2003; Oswald et al., 2000; Persson et al., 2007). A number of other techniques, such as multilocus sequence typing (MLST), multilocus variable number of tandem repeat analysis (MLVA), microarray-based comparative genomic hybridisation (mCGH) and single nucleotide polymorphism (SNP) analysis, have also emerged as interesting tools to identify genetically related STEC strains and to reveal potential epidemiological relatedness (Laing et al., 2009; Manning et al., 2008; Miko et al., 2010; Noller et al., 2003; Ziebell et al., 2008).

The use of innovative DNA-sequence typing by Bielaszewska et al. (2011) also proved crucial in the rapid identification and typing of the EHEC O104:H4 outbreak isolate, only two days after the first stool arrived in their laboratory. By generating whole-genome sequences in a short amount of time, high-throughput next-generation sequencing technologies yielded critical insights into the causative EHEC O104:H4 strain at the early stages of the German outbreak, such as its high genome sequence similarity to EAEC (Brzuszkiewicz et al., 2011; Mellmann et al., 2011; Rohde et al., 2011). They also allowed a rapid increase in the number of available *E. coli* O157:H7 genome sequences (Eppinger et al., 2011; Rump et al., 2011). Next-generation sequencing technologies will undoubtedly become increasingly popular in the production of epidemiologically relevant data during outbreaks and in the comprehension of the mechanisms underlying STEC evolution and virulence.

4. Diversity of serotypes, virulence factors and the concept of seropathotypes

The genes encoding the Shiga-toxins (*stx*) are carried by temperate lambdoid phages and therefore can be found independently of *E. coli* strains, i.e. in free phage particles (Schmidt, 2001). Thus, the prevalence of *stx* genes, detected by PCR, does not necessarily reflect the occurrence of a viable *E. coli* isolate containing those genes. However, if there is a high prevalence of *stx*-encoding phages in the farm environment these could potentially generate new STEC pathotypes by transduction (Cornick et al., 2006; Herold et al., 2004; Schmidt, 2001). The relatively low isolation rate of STEC in both animals and dairy products, suggests that such horizontal transfer of *stx*-genes by transduction takes place at a very low frequency. However, the recent large and deadly outbreak of *E. coli* O104:H4 food poisoning in Germany appears to have been caused by such a recombinant event (Scheutz et al., 2011) leading to a highly pathogenic hybrid of EHEC and EAEC (Mellmann et al., 2011) and therefore continued vigilance is necessary.

4.1. Diversity of serotypes and seropathotypes

A large number of *E. coli* serotypes produce Stx. Although numerous outbreaks worldwide have been attributed to O157:H7, more than 380 different STEC O:H serotypes have been implicated in disease (Karmali et al., 2010). Since they are associated with various levels of virulence for humans, these serotypes were classified into five seropathotypes (SPTs) based on their relative incidence in human infections and their association with outbreaks and severe disease including HUS (Karmali et al., 2003; Konczy et al., 2008; Table 2). In the absence of a consensus definition for the full set of virulence genes required for disease, this SPT-based classification was considered to be a valuable tool for a more precise molecular risk assessment of the human pathogenic potential of non-O157 STEC serogroups (EFSA, 2007).

4.2. Virulence factors

The ability of STEC to cause serious disease in humans is related mainly to their capacity to produce Stx, a family of bacteriophage-

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Table 2
Seropathotypes of STEC associated with human illness (Karmali et al., 2003).

Seropathotype	Relative incidence	Frequency of involvement in outbreaks	Association with severe disease[a]	Serotypes
A	High	Common	Yes	O157:H7, O157:NM
B	Moderate	Uncommon	Yes	O26:H11, O103:H2, O111:NM, O121:H19, O145:NM
C	Low	Rare	Yes	O91:H21, O104:H21, O113:H21, others
D	Low	Rare	No	Multiple
E	Non-human only	NA[b]	NA	Multiple

[a] HUS or haemorrhagic colitis.
[b] NA, not applicable.

encoded potent cytotoxins characterised by a high degree of diversity. Based on their antigenic differences, the Stx family has been divided into two groups, Stx1 and Stx2, each containing antigenically related members. According to the subtyping nomenclature established at the 7th International Symposium on Shiga Toxin (Verocytotoxin) — Producing *Escherichia coli* Infections (Buenos Aires, 10–13 May 2009), Stx variants include three Stx1 subtypes (Stx1a, Stx1c, Stx1d) and seven Stx2 subtypes (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, Stx2g). Stx2 is clinically the most important Stx type and the probability of HUS development in infections from strains harbouring *stx2* is higher than that of strains containing either *stx1* or both *stx1* and *stx2* (Friedrich et al., 2002). Stx2a, Stx2c and Stx2d were reported as the subtypes most commonly associated with severe outcomes of human disease (Bielaszewska et al., 2006; Persson et al., 2007).

Other important virulence determinants include the locus of enterocyte effacement (LEE) shared by EPEC. This 35–45 kb pathogenicity island is responsible for the formation of attaching and effacing (A/E) lesions on intestinal epithelial cells (Nataro and Kaper, 1998). It contains the *eae* gene encoding the outer membrane adhesin, intimin, which mediates tight contact between STEC or EPEC and intestinal epithelial cells. At least 18 intimin subtypes have been described on the basis of the variable 3′ region of the *eae* gene (Ito et al., 2007). In addition to intimin, the LEE encodes components of a type III secretion system (TTSS) which includes TTSS structural and translocator-proteins (EspA, EspB, EspD), and TTSS effector proteins (EspF-H, EspZ, Map and Tir), which are injected into human cells and subvert host cellular pathways (Garmendia et al., 2005). At least 32 other TTSS-secreted proteins displaying various functions are encoded outside the LEE within cryptic prophages and pathogenicity islands and thus usually referred to as non-LEE-encoded effectors (NLEs) (Dean and Kenny, 2009; Tobe et al., 2006).

Although the LEE island is carried by most EHEC strains, its presence is not essential for pathogenesis, and some *eae* (LEE)-negative strains (referred to as atypical EHEC) can cause HUS and occasional outbreaks (Bonnet et al., 1998; Feng et al., 2001; Karch et al., 2005; Nataro and Kaper, 1998; Paton et al., 1999). Conversely, some serotypes of LEE-positive strains isolated from cattle have never been found in humans, suggesting the importance of other virulence factors (Karmali et al., 2003).

Plasmid-encoded putative virulence factors have been described, although their role in pathogenesis remains unclear. For example, the enterohaemolysin E-hlyA, the serine protease EspP and the catalase-peroxydase KatP are encoded on a megaplasmid found in a large proportion of EHEC strains. However, the gene composition is highly variable in STEC plasmids (Brunder et al., 1999) and none of these factors seems to be critical to causing human illness because some STEC strains lacking *E-hlyA*, *espP* or *katP* have been associated with HUS.

5. STEC sources and routes to raw milk on the farm

Ruminants are the main reservoir of zoonotic STEC (Gyles, 2007), and contamination of dairy products during processing in the dairy plant is rare (Ansay and Kaspar, 1997; Kousta et al., 2010). Yet, of 193 STEC strains found in different studies at the dairy farm level, 24 were involved in human illness (Hussein and Sakuma, 2005a), which emphasises the need for control of STEC in animal reservoirs at the dairy farm. Possible mechanisms for on-farm contamination of raw milk with potentially pathogenic STEC from dairy cows, goats and ewes, are discussed below. Possible measures for preventing the contamination of raw milk and milk products at the farm will be discussed in Section 9.

5.1. STEC excretion by dairy ruminants

5.1.1. Faecal excretion

Recent reviews (Gyles, 2007; Hussein and Sakuma, 2005a) have suggested a wide range of estimates for the prevalence of healthy carriage of STEC in cattle, ranging from 0 to 71% of animals and from 0 to 100% of herds. Many of the studies that were quoted in these reviews measured the total *stx* gene prevalence in cattle, and not necessarily the isolation of a viable culture. Because *stx* genes can be present in non-pathogenic organisms, in phage, or be unassociated with a bacterium, *stx* gene prevalence estimates are an inaccurate estimation of food safety risk unless a viable culture has been obtained (EFSA, 2009a). However, vigilance for the presence of *stx* genes in foods is necessary as their transfer to *E. coli* might lead to newly emerging pathogenic STEC strains. It has also been well documented that, although many *E. coli* may contain *stx* genes, not all are pathogenic to humans (Kaper et al., 2004).

As far as O157 is concerned, one review estimates the prevalence of viable cells ranging from 3 to 8% in herds and from 0.5 to 1% in animals (Meyer-Broseta et al., 2001). According to the 2009 EFSA report (EFSA, 2009b), the average proportion of STEC positive samples, based on the investigation of faeces from 5368 animals, was 2.2%, ranging from 0% to 30%, and the average proportion of STEC O157 positive samples was 0.5%, ranging from 0% to 7.2%. A more recent and extensive study in Belgium found viable O157 isolates in 37.8% of 180 farms using a sampling technique with overshoes (Cobbaut et al., 2009). This latter result can be attributed to a better sampling technique, a specific epidemiology in the country or the improvement of the laboratory methods for detection. In general, it is probably best to keep in mind that STEC can be present, at least occasionally, on most farms (Hancock et al., 2001).

Sheep and goats are also healthy carriers of STEC. In Germany, Beutin et al. (1993) found STEC in 66.6% of 120 sheep and in 56.1% of 66 goats. In Spanish studies, 47.7% of goats from 12 herds (Cortés et al., 2005) and 36% of 1300 sheep from 93 flocks (68% were positive) carried STEC (Blanco et al., 2003). In the above studies, sheep and goats seem to carry different STEC serogroups compared to cattle, with a variety of serogroups identified. Some of these serogroups may be pathogenic to humans, but often they do not harbour the virulence genes, *stx2* and *eae* (Beutin et al., 1993; Blanco et al., 2003; Cookson et al., 2006; Kudva et al., 1996, 1997; Orden et al., 2003; Zschöck et al., 2000). Few O157 strains have been found in sheep and goats (La Ragione et al., 2009), with the exception of a Scottish study in which 42.8% of small ruminants on 14 farms were found positive for O157 (Solecki et al., 2009).

The excretion of STEC by ruminants seems to be sporadic (Heuvelink et al., 1998; Kudva et al., 1997; Mechie et al., 1997; Rahn et al., 1997), but may also be persistent over several months (Frémaux et al., 2006; Geue et al., 2009; Gyles, 2007; Hussein and Sakuma, 2005b; Liebana et al., 2005; Orden et al., 2008; Widiashi et al., 2004). Excretion varies according to the season, peaking in warmer months (Berry and Wells, 2010; Hancock et al., 1997; Heuvelink et al., 1998;

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Van Donkersgoed et al., 2001). Individual variations are also seen among cows, and may even differ between cow droppings of the same animal (McGee et al., 2004). Some cows and sheep may be 'high shedders' of O157 (Chase-Topping et al., 2008) and perhaps of other serogroups. Other factors contributing to intermittent excretion include age (Hussein and Sakuma, 2005b), diet (Jacob et al., 2009), housing (Ellis-Iversen et al., 2008; LeJeune and Wetzel, 2007; McGee et al., 2004; Vosough Ahmadi et al., 2007; Wood et al., 2007), stress (Rostagno, 2009), herd size (Erilsson et al., 2005), animal health (Byrne et al., 2003; Orden et al., 2003), geographical area (LeJeune et al., 2006) and previous contamination with STEC strains or other pathogens (La Ragione et al., 2009; Naylor et al., 2007).

5.1.2. Intra-mammary excretion

Although the major source of milk contamination by STEC is undoubtedly faecal (Hussein and Sakuma, 2005b), an intra-mammary source of STEC (pre/sub-clinical mastitis), although controversial, should not be ruled out (Lira et al., 2004; Matthews et al., 1997; Stephan and Kuhn, 1999). For example, some indication of mastitic infection with STEC was seen in a study in Switzerland by Stephan and Kuhn (1999) who found STEC in 3% of milk samples from cows with *E. coli* mastitis, and from a Brazilian study which identified the presence of the *stx* gene in 12% of 182 strains isolated from 2144 milk samples from cows with clinical and sub-clinical mastitis (Lira et al., 2004). In contrast, none of the 123 *E. coli* strains responsible for clinical mastitis cases in France, including recent isolates and some spanning 20 years, carried the *stx* gene (Raynaud et al., 2005). These observed differences between countries may be due to differences in sampling, epidemiological characteristics or antibiotic resistance patterns of the microorganisms responsible for mastitis. STEC are presumably often present on the teats in contaminated herds (Frémaux et al., 2006), and understanding the colonisation of the udder, or lack thereof, and other features exhibited by serotypes, in terms of penetration of and adhesion to the teat canal and the udder, are important.

5.2. Sources and circulation of strains on the farm

It is important to understand STEC acquisition, how such strains circulate on the farm and how the milk may subsequently be contaminated by potentially pathogenic strains.

5.2.1. Acquisition of STEC by cattle and small ruminants on the farm

5.2.1.1. Faeces and effluents. Animal-to-animal transmission is a likely contamination route of STEC within the herd, particularly when members of the herd shed STEC in high numbers (Chase-Topping et al., 2008). In addition, the introduction of newly purchased animals may be a relevant route of transmission, although there is no universal agreement on this (Cobbaut et al., 2009; Galland et al., 2001; Lahti et al., 2003; McGee et al., 2004). Environmental transmission was also shown, for example linked with poor housing conditions (Cobbold and Desmarchelier, 2002; Garber et al., 1999; Mechie et al., 1997; Smith et al., 2001; Williams et al., 2005; Wilson et al., 1993) or due to a long survival period of STEC in effluent and the environment (soil, plants, crops, grain and water), even if on-farm practices such as storage and composting may reduce STEC survival in the effluents (Fremaux et al., 2008b).

Other production animals (pigs, reindeer), pets (cats, dogs), wildlife (rabbits, deer, birds) and pests (flies, rodents) are also potential transmission vectors of STEC within ruminants, or within the farm environment (Berry and Wells, 2010; Beutin et al., 1993; Blanco et al., 2003; Erilsson et al., 2005; Lahti et al., 2003; Nielsen et al., 2004; Synge et al., 2003).

5.2.1.2. Feeds and drinking water. Contamination of feed with potentially pathogenic STEC is unusual and is more likely to occur in the feed trough than in the actual feed lot (Berry and Wells, 2010). STEC occasional presence in forage and/or concentrate can be limited by good manufacturing practices and appropriate effluent management when the feed is produced on the farm, e.g. for silage (Avery et al., 2004). Pasture can also maintain bacterial circulation by direct faeces deposit onto the ground and/or the spreading of effluents (Fremaux et al., 2008b). Drinking water can contribute to introduction or circulation of STEC within the herd, following direct or indirect faeces contamination, from bedding material, dust, in the trough or directly at the source (Cobbold and Desmarchelier, 2002; LeJeune et al., 2001; Meals and Braun, 2006; Shere et al., 1998, 2002; Van Donkersgoed et al., 2001; Vernozy-Rozand et al., 2002; Vinten et al., 2009). Furthermore, O157:H7 and perhaps other STECs can survive for long periods in water and water troughs (LeJeune et al., 2001), but environmental conditions (temperature and sun) or competitive microflora reduce bacterial numbers in water troughs and on pastures through time (Fremaux et al., 2008b).

5.2.2. Circulation of strains on and between farms

Studying the genotypic diversity of the strains that are circulating on a dairy farm is of great relevance in terms of managing STEC at the farm level. Indeed, if each contamination source is associated with one particular strain, the great diversity of strains found on farms can mean several management actions to target these different sources.

There are two hypotheses about the circulation of STECs on the farm and within the herd:

- Some researchers have found great strain diversity and multiple origins on the same farm (Cobbold and Desmarchelier, 2001; Faith et al., 1996; Frémaux et al., 2006; Galland et al., 2001; Liebana et al., 2005; Mora et al., 2004; Raynaud et al., 2005). Poly-clonality of different strains of *E. coli* coming from different mastitic animals on the same farm has been observed (Wenz et al., 2006).
- Other researchers have observed that one or a few genotypes persist on a farm, sometimes despite significant animal turnover (Lahti et al., 2003; LeJeune et al., 2004; Shere et al., 1998).

It is likely that strain circulation on the farm depends on the size of the farm, its type (feedlot, dairy farm, etc.) and farm practices. Similar strain types have been detected on distant farms, but the means by which the bacteria circulate between these distant farms is not clear (Wetzel and LeJeune, 2006).

5.2.3. Milk contamination

Generally, there are two suggested routes by which potentially pathogenic STEC can contaminate raw milk: (1) rare sub-clinical mastitis causing STEC excretion from the udder (see above); (2) faecal routes (directly or indirectly). It is proposed that lactating dairy animals carry potentially pathogenic STEC in their intestines, excrete it in their faeces, which in turn soils the teats, and the milk could be subsequently contaminated during the milking process. Therefore, limiting faecal contamination during milking is key to managing this pathogen on the farm (Hussein and Sakuma, 2005b). STEC could also potentially persist if milking equipment and pipelines are not adequately cleaned or are not well designed and not well maintained.

As STECs are commonly present in cattle, it is probably impossible to eradicate them; hence, preventive or curative measures have to be found to keep them at an acceptable level on the farm. The main pre-harvesting control measures were reviewed (Berry and Wells, 2010; LeJeune and Wetzel, 2007; Hussein and Sakuma, 2005b), with a special focus on O157:H7 for the latter two. Herd management practices aimed at reduction of faecal excretion (detection of high shedders, probiotics, vaccines, feeding changes, minimising animal stress), limitation of the circulation of the bacteria between farms, within the herd and the environment (housing, including dry bedding, pest control, limiting contact with young stock or wild animals, manure and slurry

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management), and minimisation of faecal contamination during milking will help to reduce milk contamination. No distinction between curative control measures taken on a given farm after isolation of a potentially pathogenic STEC strain in the milk, and preventive measures concerning other farms on routine production, was made. This distinction should be considered for further research. Both curative and preventive measures have to be adapted to each specific farm situation and they need to be better evaluated (efficiency) and prioritised, alone or in combination, in different farming systems. Indeed, since a lot of data on STEC at the farm level has come from work on feedlots, more research on dairy farms of different types would be useful. Some of these measures are already in use on raw milk producing farms, for example through guide to good hygiene practices.

Preventive measures for raw milk producing farms must also take into account the preservation of complex microbial ecosystems of the farm, which contain bacteria, yeasts or moulds that are essential for raw milk cheese-making (lactic or ripening bacteria for example) and that may be important in the control of potentially pathogenic STEC as it was shown for other pathogen bacteria (Millet et al., 2006; Raynal-Ljutovac et al., 2006, 2008).

In many countries, quantitative risk assessment and other modelling studies lack data at the farm level (Berry and Wells, 2010), making predictions of STEC in this regard difficult and unreliable (Delignette-Muller et al., 2008). Studies on the prevalence and enumeration of serotypes other than O157 are needed, but due to the generally low numbers of STEC, better methodology is required for enumeration.

6. Occurrence of STEC in raw milk and raw milk cheeses

In recent years, DNA based methods have been used to detect genes encoding pathogenicity and virulence factors, such as *stx1*, *stx2*, *eae* and E*hlyA*, in *E. coli* strains and have become a supplement to traditional culturing and serogrouping of STEC. However, it is important to realise that detection of genes in food does not necessarily imply the occurrence of a viable isolate: recovery of a viable culture is still necessary to categorically state that a sample was 'positive' for a potentially pathogenic strain. It is therefore important to clearly distinguish between 'prevalence' of genes and 'occurrence' of viable strains. In addition, studies on occurrence are not always comparable due to differences in the methodology used for recovery of STEC from food. For milk and cheese, standard and validated methods are available for detection and isolation of STEC O157. However, for the other serogroups there are no universally accepted and validated methods, but pragmatic and different approaches have been applied in different studies.

6.1. Occurrence of STEC in raw milk

The significance of *E. coli* O157:H7 and other STEC for the dairy sector has been previously reviewed (Bastian and Sivelä, 2000; Hussein and Sakuma, 2005b). The occurrence of STEC in raw milk has not changed dramatically over the last 10 years, and is typically between 0 and 2%. Since 2000, the introduction of DNA based methods and the production of additional data from countries and regions outside Europe and US, including the incidence of serogroups other than O157, have made the picture more complex. Several studies have included only a limited number of samples, thus adding to this complexity. These data suggest geographical variations in occurrence of various sero- and genotypes, and that goats and sheep may be reservoirs of STEC in some parts of the world. Virulence gene prevalence in raw milk samples (*stx1* and/or *stx2*) is also in general significantly higher than isolate occurrence (Table 3). Studies from Ireland and USA, showed that virulence gene prevalence in raw milk was 36% and 21%, respectively, while the occurrence of isolates was 0.8% and 3.2%, respectively (Cobbold et al., 2008; Lynch et al., 2012). Recent studies also show high prevalence of *stx* genes in milk filters (Van Kessel et al., 2011). Results from selected studies on the occurrence of STEC isolates and prevalence of *stx* genes in raw milk are presented in Table 3.

6.2. Occurrence of STEC in cheeses and other dairy products

A selection of recent studies on the occurrence of STEC in cheeses and other dairy products (not including milk) is presented in Table 4. Most of the studies focus on cheeses made from raw/unpasteurised milk. Several of these studies have limitations, mainly due to a small number of samples (typically less than 100). The most comprehensive studies on the occurrence of STEC or prevalence of *stx* in cheese are published in Europe (Coia et al., 2001; Conedera et al., 2004; Pradel et al., 2000; Rey et al., 2006; Stephan et al., 2008; Vernozy-Rozand et al., 2005a; Zweifel et al., 2010). A few dairy products other than cheeses, including cream, ice-cream, yoghurt and butter, have also been studied for the occurrence of STEC. Some studies also indicate that STEC isolates from dairy products in most cases differ from the classical pathogenic genotypes, harbouring *stx1* rather than *stx2* (Pradel et al., 2008). Other studies contradict this (Zweifel et al., 2010).

7. STEC human infection and the main outbreaks associated with milk and dairy products

7.1. STEC in humans

The estimated infectious dose of STEC that can cause an outbreak is very low. A dose of pathogenic *E. coli* O157:H7 of 5–50 viable cells (Betts, 2000) can cause illness. Delignette-Muller et al. (2008) showed that 3×10^3 cells may affect 50% of exposed children under five years of age. The symptoms associated with STEC infection in humans vary from mild to bloody diarrhoea, which is often accompanied by abdominal cramps, usually without fever. STEC infections can result in HUS which is characterised by acute renal failure, anaemia and lowered platelet counts. HUS develops in up to 10% of patients infected with STEC O157 and is the leading cause of acute renal failure in young children. In Europe, in 2009, a total of 242 confirmed STEC cases developed HUS. The STEC serogroup O157 was identified in 47% of reported cases in 0–4 year old children and the STEC serogroup O26 in 15% of these cases (EFSA, 2011b).

Non-O157 infections may also induce a range of other illnesses, from mild gastroenteritis to critical illness, and death, either as sporadic cases or in outbreaks. Due to inadequate analytical methods and epidemiological and laboratory surveillance, non-O157 STEC infections may be under-recognised and under-reported around the world (Hanna et al., 2010).

Reports from public health surveillance studies worldwide indicate that sporadic cases of infection with STEC greatly outnumber outbreak cases (EFSA, 2011b). Strains of O157 cause the majority of disease, but in recent years, improved diagnostic assays for non-O157 serogroups have contributed to an increased appreciation of the incidence of disease caused by non-O157 STEC. In the United States, Johnson et al. (2006) evaluated the emerging clinical importance of non-O157 STEC and concluded that these strains may account for up to 20 to 50% of all STEC infections.

In Europe, the total number of confirmed cases reported in 2009 was 3573 corresponding to an overall notification rate of STEC infection reported by the 25 member states of 0.75 cases per 100,000 population. More than half (51.7%) of reported confirmed human STEC infections were associated with the O157 serogroup and 5.4% with O26 serogroup (EFSA, 2011b). Over the period 2002–2006, more than one-third of STEC illnesses were attributed to non-O157 STEC, with 20% due to five serogroups, i.e. O26, O103, O91, O145 and O111 (EFSA, 2007). Except for O91, these serogroups also accounted for the

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Table 3
Selected studies reporting on the occurrence of STEC and prevalence of *stx* genes in raw milk.

Region/country	Sample analysed	STEC[a] isolate occurrence (%) and/or *stx* gene prevalence (%) (N = number of samples)	Reference
Africa			
Kenya	Raw cow's milk (households)	0.8% (N = 432) *E. coli* O157:H7. One *stx1* positive strain	Arimi et al. (2005)
	Raw cow's milk (households)	0% (N = 260) *E. coli* O157:H7	Kangethe et al. (2007)
America			
Argentina	Raw cow's milk (bulk tank)	0% (N = 150) *E. coli* O157:H7	Roldán et al. (2007)
Brazil	Raw cow's milk (cattle with mastitis)	12.08% (N = 2144) positive for a combination of *hly*, *stx1*, *stx2* and/or *eae* gene	Lira et al. (2004)
USA	Raw cow's milk (bulk tank)	0.2% (N = 859) *E. coli* O157:H7	Karns et al. (2007)
	Raw cow's milk (for cheesemaking)	0% (N = 67) *E. coli* O157:H7	D'Amico et al. (2008).
	Raw sheep's milk (for cheesemaking)	0% (N = 22) *E. coli* O157:H7	D'Amico et al. (2008).
	Raw goat's milk (for cheesemaking)	0.75% (N = 49) *E. coli* O157:H7	D'Amico et al. (2008).
	Raw cow's milk (bulk tank)	2.4% (N = 248) STEC	Jayarao et al. (2006)
	Raw cow's milk (bulk tank)	3.8% (N = 131) *E. coli* non-O157 harbouring *stx2* or *stx1*	Jayarao and Henning (2001)
	Raw cow's milk (bulk tank)	3.2% (N = 531) STEC	Cobbold et al. (2008)
	Raw cow's milk (bulk tank)	0.75% (N = 268) *E. coli* O157	Murinda et al. (2002)
	Raw cow's milk (bulk tank)	15.2% (N = 536) of samples positive for *stx1* and/or *stx2*	Van Kessel et al. (2011)
	Milk filters	51% (N = 519) of samples positive for *stx1* and/or *stx2*	Van Kessel et al. (2011)
Trinidad	Raw cow's milk (bulk tank at collection centres)	0.8% (N = 380) *E. coli* O157	Adesiyun et al. (2007)
	Raw cow's milk (bulk tank and other sources)	0% (N = 933 *E. coli* isolates) STEC from bulk tank milk	Roopnarine et al. (2007)
Asia			
Turkey	Raw cow's milk (individual animals)	1% (N = 100) *E. coli* O157	Oksuz et al. (2004)
India	Raw cow's milk and milk products	1.8% (N = 553) *E. coli* O157	Sehgal et al. (2008)
China	Raw cow's milk	0% (N = 209) *E. coli* O157:H7	Chao et al. (2007)
Europe			
EU	Raw cow's milk[b]	1.7% (N ~ 1300) in 2008 0.5% (N = 1079) in 2007 0.4% (N = 3474) in 2006 1.4% (N = 3947) in 2005	EFSA (2010)
Spain	Raw goat's and sheep's milk (bulk tank)	10.8% (N = 360) STEC 0.3% (N = 360) *E. coli* O157	Rey et al. (2006)
Scotland	Raw cow's milk	0% (N = 500) STEC	Coia et al. (2001)
Northern Ireland	Raw cow's milk (bulk tank)	2.14% (N = 420) STEC (harbouring *stx1*, *stx2* and/or *eae*)	McKee et al. (2003)
Ireland	Raw milk (bulk tank)	10% (N = 20) *E. coli* O157 (*stx* negative)	Murphy et al. (2007)
	Milk filters	3.1% (N = 161) *E. coli* O157 (*stx* positive) 2.8% (N = 161) *E. coli* O26 (*stx* positive)	
	Raw cow's milk	36% (N = 120) of samples positive for *stx*1and/or *stx2* 0.8% (N = 120) of samples positive for *E. coli* O157, O111, O26, O103 and/or O145	Lynch et al. (2012)
	Milk filters	69% (N = 117) of samples positive for *stx*1and/or *stx2* 1.6% (N = 117) of samples positive for *E. coli* O157, O111, O26, O103 and/or O145	
Netherlands	Raw cow's milk (individual animals)	0% (N = 140) *E. coli* O157	Schouten et al. (2005)
	Milk filters	0% (N = 4) *E. coli* O157	
	Raw cow's milk (bulk tank)	0% (N = 13) *E. coli* O157	
Italy	Raw cow's milk (retailer)	0.6% (N = 157) *E. coli* O126 (*stx1* and *stx2* positive, lacking *eae*) and non-O157	Pontello et al. (2003)
	Raw buffalo milk	0.6% (N = 160) *E. coli* O26 (*stx1* and *stx2* positive)	Lorusso et al. (2009)
	Raw goat's milk	1.7% (N = 60) *E. coli* O157:H7	Foschino et al. (2002)
	Raw cow's and goats milk	0.14% (N = 144) *E. coli* O157	Picozzi et al. (2005)
	Raw cow's milk	0% (N = 994) STEC	EFSA (2011a)
Greece	Raw cow's milk	2.2% (N = 950) *E. coli* O157	Solomakos et al. (2009)
	Raw goat's milk	0.7% (N = 460) *E. coli* O157	
	Raw sheep's milk	0.8% (N = 595) *E. coli* O157	
	Raw sheep's milk	1% (N = 100) *E. coli* O157:H7	Dontorou et al. (2003)
	Raw goat's milk (individual animals)	12.6% (N = 87) *E. coli* O157:H7	
	Raw sheep's milk (individual animals)	13.8% (N = 29) *E. coli* O157:H7	Zdragas et al. (2009)
Switzerland	Raw cow's milk	0% (N = 310) STEC	Stephan and Buehler (2001)
	Raw goat's milk (bulk tank)	16.3% (N = 344) of samples positive for *stx1* and/or *stx2*	Muehlherr et al. (2003)
	Raw sheep's milk (bulk tank)	12.7% (N = 63) of samples positive for *stx1* and/or *stx2*	
France	Raw cow's milk	15% (N = 778) of samples positive for *stx1* and/or *stx2*	Raynaud et al. (2006)
	Raw cow's milk	4.8% (N = 205) of samples positive for *E. coli* O26, O103, O111, O145 or O157 Genetic markers	Perelle et al. (2007)
	Raw cow's milk	21% (N = 205) of samples positive for *stx1* and/or *stx2* 3.4% (N = 205) of samples positive for *E. coli* O91:H21 Genetic markers	Madic et al. (2009)
Germany	Raw cow's milk	0% (N = 209) STEC	Messelhäusser et al. (2008)
	Raw cow's milk	2.5% (N = 403) STEC	Zweifel et al. (2006)
	Raw cow's milk (monitoring at farm)	1.5% (N = 337) STEC other than *E. coli* O157	EFSA (2011b)
Belgium	Raw cow's milk	0.7% (N = 143) *E. coli* O157.H7	De Reu et al. (2004)
The Czech Republic	Milk filters	2% (N = 192) *E. coli* O157:H7	Čížek et al. (2008)
Slovakia	Raw cow's milk	0.4% (N = 269) *E. coli* O157	EFSA (2011a)

[a] Culture positive sample with strain harbouring *stx1* and/or *stx*2 unless otherwise commented.
[b] Most data aimed at detecting *E. coli* O157. Only a few investigations conducted with analytical methods aiming at detecting all serotypes of STEC.

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Table 4
Selected studies reporting on the occurrence of STEC and prevalence of *stx* genes in cheeses and other dairy products.

Region/country	Sample analysed	STEC[a] isolate occurrence (%) and/or *stx* gene prevalence (%) (N = number of samples)	Reference
America			
Peru	Soft cheese made from raw milk	7.8% (N = 102) *E. coli* O157	Mora et al. (2007)
Venezuela	Telita cheese	2% (N = 100) *E. coli* O157	Márquez and García (2007)
Asia			
Turkey	White pickled cheese made from raw milk	4% (N = 50) *E. coli* O157	Oksuz et al. (2004)
Europe			
EU	Cheese made from raw milk	0.2% (N = 2876) STEC in 2005 2.4% (N = 1064) STEC in 2006 0.5% (N = 1961) STEC in 2007 1.8% (N ~ 700) STEC in 2008	EFSA (2010)
Belgium	Cheese made from raw cow's milk	5.6% (N = 71) *E. coli* O157:H7	De Reu et al. (2002)
	Cheese made from raw goat's and sheep's milk	0% (N = 222) *E. coli* O157	Imberechts et al. (2007)
	Butter and cream made from raw milk	0% (N = 181) *E. coli* O157	
	Butter, yoghurt, cheese, ice cream and fresh cheese made from raw milk	0% (N = 64, N = 9, N = 16, N = 7, N = 4 resp.)	De Reu et al. (2004)
Italy	Cheese made from raw milk	0% (N = 143) *E. coli* O157	Civera et al. (2007)
	Cheese made from pasteurised milk	0% (N = 60) *E. coli* O157	
	Mozzarella cheese made from raw buffalo's milk	0% (N = 93) *E. coli* O157	Martucciello et al. (2008)
	Dairy products from pasteurised cow's milk	0% (N = 657) *E. coli* O157	Conedera et al. (2004)
	Dairy products from raw cow's milk	0% (N = 811) *E. coli* O157	
	Dairy products from pasteurised sheep's milk	0% (N = 477) *E. coli* O157	
	Dairy products made from raw sheep's milk	0% (N = 502) *E. coli* O157	
	Mozzarella cheese made from buffalo milk	0% (N = 501) *E. coli* O157	
Portugal	Cheese made from raw cow's, sheep's and goat's milk	0% (N = 70) *E. coli* O157	Almeida et al. (2007)
Spain	Dairy products from raw ovine and caprine milk (milk, cheese curd, cheese)	1.8% (N = 502) STEC (45 *stx* + samples)	Rey et al. (2006).
	Fresh cheese curds (ovine/caprine)	0% (N = 103) STEC (4 *stx* + samples)	
	Cheese	0% (N = 39) STEC (2 *stx* + samples)	
	Raw ewe's Castelano cheese (long-ripened, hard cheese)	2.4% (N = 84) STEC	Caro and Garcia-Armesto (2007)
Scotland	Cheese made from raw cow's milk	0% (N = 739) *E. coli* O157	Coia et al. (2001)
Switzerland	Cheese (semihard, hard and soft) made from raw cow's and goat's milk	2% (N = 796) STEC (4.9% stx + samples)	Stephan et al. (2008)
	Cheese (semihard, hard and soft) made from raw cow's, goat's and ewe's milk	1.9% (N = 1502) STEC (5.7% stx + samples)	Zweifel et al. (2010)
France	Cheese made from raw milk	11.7% (N = 180) STEC(30.5% stx + samples)	Fach et al. (2001)
	Cheese (soft, hard, unripened, blue mold) made from raw milk	13.1% (N = 1039) STEC	Vernozy-Rozand et al. (2005a)
	Cheese	1% (N = 603) STEC (10% *stx* + samples)	Pradel et al. (2000)
	Cheese made from raw milk	27.7% (N = 112) *stx* + samples	Auvray et al. (2009)
	Cheese made from raw milk	5.5% (N = 400) STEC, including 1.8% STEC O26:H11 (29.8% *stx* + samples)	Madic et al. (2011)
Germany	Cheese made from raw cow's milk	0.48% (N = 209) STEC	Messelhäusser et al. (2008)

[a] Culture positive sample with strain harbouring *stx1* and/or *stx2* unless otherwise commented.

majority of non-O157 serogroups associated with HUS (Table 5). The distribution of STEC infections in 2009, as in previous years, followed a seasonal pattern, with a rise in case counts over the summer and autumn months, peaking in September (EFSA, 2011b). Regional differences may also occur; for example, STEC O157 is more frequently linked to human infections in the UK and Ireland than in continental Europe where non-O157 STEC serogroups predominate.

Table 5
The most commonly reported STEC serogroups amongst EU member states over the period 2002–2006 for human and HUS cases in which serogroups were established.

	All human cases		HUS cases	
	No.[a]	%	No.[a]	%
O157	7227	66.0	310	68.3
O26	732	6.7	52	11.5
O103	603	5.5	20	4.4
O91	425	3.9	1	0.2
O145	312	2.8	27	6.0
O111	180	1.6	18	4.0
O146	153	1.4	0	0.0
O128	93	0.8	1	0.2
O55	74	0.7	3	0.7
Other	1199	10.9	22	4.9
Total	10,998		454	

[a] Each value represents the sum, for each serogroup, of the numbers of cases reported each year over the period 2002–2006 (EFSA, 2007).

7.2. Different vehicles of human infection

The primary vehicles of transmission are associated with ruminants, which are the main reservoir of STEC. They include contact with animals or their environments and consumption of contaminated food and water (Table 6), although person–person contact has been reported as the cause of about 29% of outbreaks (Kaspar and Doyle, 2009). The foodborne route often involves consumption of undercooked meat, water, dairy products made from raw milk or post-processing contamination of raw or under cooked vegetables and ready-to-eat food. In 18.8% of non-O157 outbreaks, no vehicle was identified.

The latest data from EFSA (EFSA, 2011b) shows that bovine meat is believed to be a major source of foodborne STEC infections for humans. Raw cow's milk is also considered a source of human STEC infections in Europe. In North America, a large number of outbreaks of STEC O157 infection have been attributed to vegetables (CDC, 2006; Hilborn et al., 1999; Wendel et al., 2009).

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Table 6
Comparison of the relative importance of transmission vehicles associated with outbreaks of *E. coli* O157:H7 and non-O157:H7 STEC infection.
From Kaspar and Doyle (2009).

Vehicle	Non-O157:H7 STEC	*E. coli* O157:H7
Animal contact	6.2%	9.7%
Water	10.0%	25.6%
Person–person contact	28.8%	6.8%
Dairy	10.0%	12.5%
Meat	11.2%	24.6%
Produce	6.2%	9.2%
Other food	8.8%	5.8%
Unknown	18.8%	5.8%

7.3. Foodborne STEC infections and outbreaks

When interpreting data on the occurrence of STEC from food or animals, it is important to remember that, due to differences in sampling strategies and analytical methods used, data from different investigations are often not directly comparable, especially those between countries and different years. Furthermore, the most widely used analytical method only aims at detecting *E. coli* O157, and there are relatively few investigations aimed at detecting other STEC serogroups.

Table 7 shows the STEC outbreaks that have been linked to milk and dairy products. Raw milk consumption (cow's and goat's milk) has been associated with several cases in Europe, USA and Canada. Most of these were associated with *E. coli* O157, although other serotypes or serogroups, including O22:H8, O110:H$^-$, O80:H$^-$, and O145 have also been identified as causative agents. Consumption of contaminated soft and semi-soft cheeses has also been implicated in outbreaks, especially those made from unpasteurised cow's and goat's milk. *E. coli* O157:H7 was linked to the majority of cases, but O27:H20, O103, O26, O145, O119:B14, O27:H20, and O104:H21 have also been implicated. The number of reported outbreaks due to non-O157 STECs remains relatively low, probably due to lack of investigation and also to lower pathogenicity.

A number of STEC outbreaks associated to pasteurised milk (Upton and Coia, 1994) and cheese (Nooitgedagt and Hartog, 1988) have been reported. These were probably due to defective pasteurisation and/or post processing contamination. Farm yoghurt, farm ice-cream and milk shakes made with pasteurised milk have also been associated with STEC outbreaks (Becker, 2005; De Schrijver et al., 2008; Morgan et al., 1993). A HUS-outbreak (5 cases) caused by O145:H28 and O26:H11, and associated with dairy farm ice-cream made from pasteurised milk has been recently described (Buvens et al., 2011). Indistinguishable isolates from patients, ice-cream leftovers and environmental farm samples (hay, dust, calf faeces, overshoes) were found. This outbreak was probably the result of a post-pasteurisation contamination of the ice-cream manufactured at the dairy farm. It was estimated that approximately 400 cells of STEC O145:H28 had been ingested by the patients (the concentration of STEC O26 was 100-fold lower in the ice-cream leftovers).

8. Survival of STEC during cheesemaking

There are many varieties of cheeses produced which are categorised into four types: soft, semi-soft, semi-hard and hard cheeses. The different processing technologies used in the various cheeses can influence the survival of *E. coli*. While the processing technology may create conditions that are unsuitable for growth of *E. coli*, they may not cause inactivation and survival may still be an issue. Peng et al. (2011) have reviewed stress response mechanisms important for allowing STEC to survive raw milk cheese production. Some cheeses, however, particularly soft and semi-soft cheeses, are prone to surface contamination where ripening can result in changing physico-chemical characteristics that may allow greater survival or even growth. Thus, while control of the cheesemaking process is important, process control during ripening is equally important.

8.1. Factors affecting growth and survival of STEC during cheesemaking

The microbial stability of cheese is determined by the combined application of different microbial hurdle factors (low pH, a_w values, NaCl, starters) which become more and more challenging during the manufacturing process (Leistner, 1995). Nevertheless, several studies have shown that the application of each individual hurdle factor may not eradicate STEC. Different authors have demonstrated that survival of *E. coli* strains can be enhanced by cross-protection when subjected to combinations of stresses such as acid, salt and heat stress (Rowe and Kirk, 1999). But it can be conjectured that a synergistic effect of these different hurdle factors exists in cheese (Gálvez et al., 2007) and that the sequence of application of different hurdles is important. The majority of studies on growth/survival of STEC in cheese have been undertaken on *E. coli* O157:H7 and little is known about non-O157 STEC strains.

8.1.1. pH

During the cheesemaking process, lactic acid bacteria produce acids that lead to a decrease in the pH of the cheese which can reach values as low as 4.5 (e.g. lactic cheeses, cream cheeses, or Feta; Mahaut et al., 2000). Several studies have shown that *E. coli* O157:H7 has an optimum growth at pH of around 7 and is able to grow in a pH range between 4.5 and 9 (Dineen et al., 1998; Glass et al., 1992). Furthermore, some strains of *E. coli* O157:H7 are acid-resistant, being able to withstand pH values as low as 3.0 (Jordan et al., 1999). Thus, due to clearly defined acid resistance systems (Foster, 2004), the lowest pH of cheese will not inactivate *E. coli* O157:H7 or other STEC. Acid adaptation to the non-lethal pH in the cheese may promote greater survival of *E. coli* O157:H7 during passage through the acid environment of the stomach (Jordan et al., 1999).

8.1.2. Temperature

In cheese processing, pasteurisation of milk is an effective treatment for inactivating pathogens, including STEC. Early studies revealed that the pasteurisation of milk at 72 °C for 16 s was sufficient to eliminate *E. coli* O157:H7 (D'Aoust et al., 1988). Therefore, the significance of STEC in cheese refers to raw milk cheese or situations where there was pasteurisation failure or post-pasteurisation contamination (Baylis, 2009). For Mozzarella cheese, heating processes during manufacture (80 °C for 5 min) will inactivate STEC (Spano et al., 2003). The effect of sub-pasteurisation heat treatment of milk, however, can have varying effects such as induction of an adaptive response (Usajewicz and Nalepa, 2006). Moreover, the fat molecules may protect STEC during such heat treatment (Erickson and Doyle, 2007; Kaur et al., 1998). The ripening temperature may influence survival, however, *E. coli* O157:H7 was shown to survive for 60 to 75 days, respectively, in Camembert and Feta cheeses at 2 °C (Ramsaran et al., 1998). The same survival results were observed in Cheddar for 60 days at 7 °C (Schlesser et al., 2006).

8.1.3. Water activity (a_w) and salting

During cheese manufacturing, a salting step is usually applied to provide a supplementary drainage of whey, add to the flavour characteristics, help the rind formation and to regulate the a_w in order to control microbial growth (Mahaut et al., 2000). The minimum a_w for growth of *E. coli* is 0.945–0.95 (Lindblad and Lindqvist, 2010; Salter et al., 2000; Sperber, 1983). It has also been shown that in TSB (tryptone soya broth), *E. coli* O157:H7 was inhibited by NaCl at concentrations ≥8.5 g/100 g but that it could grow in the presence of NaCl at 6.5 g/100 g (Glass et al., 1992).

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Table 7
STEC outbreaks associated with milk and dairy products.

Year	Dairy product	Serotype, virulence factors	Number of cases	Country	References
1986	Raw milk	O157:H7	2 HUS	USA	Martin et al. (1986)
1989	Raw milk	O22:H8	1 HUS	Germany	Bockemühl et al. (1990)
1990	Raw milk	O157:H7	2 HUS	USA (Washington)	Wells et al. (1991)
1991	Farm yoghurt (pasteurised milk)	O157:H7	16–5 HUS	UK (England and Wales)	Morgan et al. (1993)
1992–1993	Farm fresh goat's and cow's milk cheese (raw milk)	O119:B14, VT2	4 HUS	France (Cher)	Casenave et al. (1993), Deschênes et al. (1996)
1992–1993	Raw milk	O157:H7	14–0 HUS	USA (Oregon)	Keene et al. (1997)
1993	Milk	O157	7–3 HUS	UK (England and Wales)	Ammon (1997), Wall et al. (1996)
1994	Pasteurised milk	O157:H7, Vt2 + PT2	>100–9 HUS	UK (Scotland)	Upton and Coia (1994)
1994	Farm raw milk cheese	O157, VT2, PT28	22–1 HUS	UK (Scotland)	Ammon (1997), Strachan et al. (2006)
1994	Milk	O157, VT2	8–3 HUS	UK (Scotland)	Ammon (1997)
1994	Farm fresh goat's milk cheese (raw milk)	O103	4 HUS	France (Ardèche)	Decludt (1995)
1994	Milk (post pasteurisation contamination suspected)	O104:H21	11 confirmed, 7 suspected	USA (Montana)	CDC[2] (1995b)
1995	Farm raw goat's milk	O157:H7	4 HUS	Czech Republic	Bielaszewska et al. (1997)
1996	Raw milk cheese	O110:H$^-$	3–0 HUS	Germany	Bockemühl and Karch (1996)
1996	Milk	O157	12–1 HUS	UK	Clark et al. (1997)
1997	Unpasteurised milk	O157	6	Finland	Lahti et al. (2002)
1997	Unpasteurised milk	O157	3 HUS	Finland	Lahti et al. (2002)
1997	Unpasteurised milk	O157	2 HUS	Finland	Lahti et al. (2002)
1997	Cheese	O157	5 (2 confirmed)	UK	Strachan et al. (2005)
1998	Unpasteurised milk	O157	17	Finland	Lahti et al. (2002)
1998	Unpasteurised farm cheese	O157	4	UK (Scotland)	Strachan et al. (2006)
1998	Raw milk cheese	O157, PT2, VT2	10–1 HUS	UK (Dorset)	CDSC[1] (1998b)
1998	Unpasteurised cream	O157:H7	7	UK (England)	CDSC[1] (1998a)
1998	Fresh cheese curds	O157:H7	55–2 HUS	USA (Wisconsin)	CDC[2] (2000)
1999	Farm milk	O157	38–3 HUS	UK (North Cumbria)	Anonymous (1999)
1999	Homemade goat's milk cheese (raw milk)	O157, PT 21, PT 28	27–1 HUS	UK (Scotland)	Curnow (1999)
1999	Raw milk	O157	3	UK (Scotland)	Strachan et al. (2006)
2000	Raw milk	O157	2	UK (Scotland)	Strachan et al. (2006)
2001	Raw goat's milk	O157:H$^-$, sorbitol + Stx2+, Stx2c+	2–1 HUS	Austria	Allerberger et al. (2001)
2001	Goat's milk	O157:H7	5–2 HUS	Canada (British Columbia)	McIntyre et al. (2002)
2002–2003	Raw milk gouda	O157:H7	13–2 HUS	Canada (Alberta)	Honish et al. (2005)
2003	Raw cow's milk	O26:H$^-$, stx2+, eae + hly +	2 HUS	Austria	Allerberger et al. (2003)
2003	Raw cow's milk	O157, stx2+, eae + ehxA +	9–4 HUS (family outbreak)	Slovakia	Liptakova et al. (2004)
2003–2004	Organic milk	O157:H$^-$	25–0 HUS	Danemark	Jensen et al. (2006)
2004	Raw milk cheese	O157:H7	3	Canada (Quebec)	MAPAQ[3] (2004)
2004–2006	Farm fresh goat's cheese (raw milk)	O157, stx2+, eae +	2 HUS	France	INVS[4] (2004), Espié et al. (2006)
2005	Milk	O157:H7	6	Canada (Ontario)	Honish et al. (2005)
2005	Milk shake	O157:H7	16–1 HUS	Canada (Alberta)	Becker (2005)
2005	Raw milk	O157:H7	18–4 HUS	USA (Washington–Oregon)	CDC[2] (2007a)
2005	Raw milk cheese (brie)	O26, O80, stx2 +, eae +	6 HUS	France (Normandie)	INVS[4] (2007)
2006	Raw milk	O80:H$^-$, stx1+, eae + hly+, O145, stx1+, stx2+ eae +	59–1 HUS	Germany	RKI[5] (2008)
2006	Raw cow's milk	O157:H7	2 HUS	USA (California)	Buglino et al. (2008)
2006	Organic raw milk and cow's colostrum	O157:H7, other STEC serotypes	6–2 HUS	USA (California)	CDC[2] (2008)
2007	Farm ice cream	O145:H28, stx2+, eae + ehxA + O26:H11, stx1+, eae + ehxA +	12–5 HUS	Belgium	De Schrijver et al. (2008), Buvens et al. (2011)
2007	Pasteurised cheese	O121, O26, O84	135–10 HUS	USA (Colorado)	CDC (2007b)
2008	Raw goat's milk	O157:H7	4–1 HUS	USA (Missouri)	Marler (2009)
2009	Raw milk	O157:H7, O121	3	USA (Washington)	Food Poison Journal (2009)
2010	Raw milk	O157:H7	8–1 HUS	USA (Minnesota)	Minnesota Department of Health, 2010
2010	Raw goat's milk	O157:H7	30–2 HUS	USA (Colorado)	Marler (2010)
2010	Gouda cheese	O157:H7	38–1 HUS	USA (5 states)	CDC[2] (2010)
2010	Raw milk cheese	O157:H7	8	USA (4 states)	Rothschild (2010)

Stx = VT Shiga-toxin (= verocytotoxin)
stx Shiga-toxin gene
HUS Haemolytic Uremic Syndrome
1— CDSC Communicable Disease Surveillance— USA
2— CDC Centers for Disease Control and Prevention— USA
3— MAPAQ Ministère de l'Agriculture, des pêcheries et de l'Alimentation du Quebec
PT Phage Type
4— INVS Institut de Veille Sanitaire — France
5— RKI Robert Koch Institut.

8.1.4. Anti-microbial interactions

Milk microflora as well as starter lactic acid bacteria may act antagonistically against STEC strains (Dineen et al., 1998), although little is understood about the mechanisms of these interactions. The specific antimicrobial mechanisms of lactic acid bacteria that could inhibit pathogenic *E. coli* include the production of organic acids, hydrogen peroxide, carbon dioxide, diacetyl and production of bacteriocins (De Vuyst and Vandamme, 1992).

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Little is known about interactions with other STEC serogroups, or between *Penicillium* spp. and STEC in blue cheeses or with propionibacteria.

8.2. Behaviour of STEC during cheesemaking

8.2.1. Survival/growth during cheese manufacture

If present in cheese, STEC will generally be at low numbers. However, these low numbers are difficult to detect (Jordan and Maher, 2006) and experiments are often undertaken with higher inoculation levels (Montet et al., 2009; Ramsaran et al., 1998; Reitsma and Henning, 1996). In addition, it is difficult to compare results obtained from different studies as many authors use different protocols for analysis.

At the initial stages of cheesemaking, the temperature (30 °C) and a_w value (0.99) of milk are ideal for the growth of *E. coli* O157:H7 and other STEC. During the transition from milk (liquid) to curds (solid), there is an approximately 10-fold increase in the numbers of *E. coli* O157:H7 due to the entrapment of the pathogen in the curds after whey drainage. As CFU/mL for milk and CFU/g for cheese are often plotted on the same graph (Morgan et al., 2001; Papageorgiou and Marth, 1989; Ryser and Marth, 1987a, 1987b), any apparent increase in numbers could be due to growth or cell concentration during curd formation — it is not possible to distinguish between these two phenomena. By plotting all counts in similar units of CFU/g dry weight, Maher et al. (2001) demonstrated actual growth of *E. coli* O157:H7 in a semi-soft laboratory scale cheese during the initial stages of manufacturing. Most authors have reported an increase in numbers of STEC during the initial phase of cheesemaking, however in the absence of consistent units on the graph, this increase could be due to either growth of *E. coli* O157:H7 during cheesemaking, or to concentration of the bacteria during curd formation. In soft cheese such as Feta and Telemes, studies have shown (Govaris et al., 2002) that after inoculation of O157:H7 into pasteurised ewe's and cow's milk, an increase in the concentration of the STEC during the first 10 h of manufacturing was observed. Hudson et al. (1997) also observed that 3 h after curd formation, counts of *E. coli* O157:H7 increased by 1 log CFU/g in the curds of Feta cheese made from cow's milk. Ramsaran et al. (1998) have shown similar behaviour of *E. coli* O157:H7 during manufacture of Feta and Camembert in raw and pasteurised milk by using nisin producing starters. Montet et al. (2009) showed that in Camembert, after inoculation of 3 log CFU/mL of non-*E. coli* O157:H7 STEC in milk, STEC increased by 1 to 2.2 log CFU/g during the first steps of the cheese manufacturing. Hudson et al. (1997) reported that *E. coli* O157:H7 increased during the manufacture of Colby, Cottage, and Romano cheeses, while Wahi et al. (2006) reported that *E. coli* O157:H7 decreased rapidly, but some cells survived during the manufacturing process of Paneer, which is a popular cheese in India.

8.2.2. Survival during cheese ripening and storage

As mentioned earlier, during ripening and storage, the behaviour of the STEC can be affected by the microbial hurdles found in cheese. Generally, there is a decline in the numbers of *E. coli* O157:H7 during cheese ripening, although the rate of this decline will vary. In Feta cheese and Telemes, *E. coli* O157:H7 was not detectable after 44 and 36 days ripening, respectively (Govaris et al., 2002), although Ramsaran et al. (1998) observed that, with an inoculation level of 10^4 CFU/mL, the pathogen population in the cheese was more than 10^6 CFU/g after 75 days of refrigerated storage. Hudson et al. (1997) observed a 3-log cycle reduction in counts of *E. coli* O157:H7 after 27, 30, and 27 days for Colby, Romano, and Feta cheeses, respectively.

Reitsma and Henning (1996) produced Cheddar cheese from milk contaminated with high (10^3 CFU/mL) and low (1 CFU/mL) inocula of *E. coli* O157:H7, and studied the fate of the pathogen in the cheese during ripening at 4 °C. They found that the pathogen survived for 158 days for the high inoculum trial, but it was not detectable after 130 days for the low inoculum trial. In raw goat's milk cheeses, Vernozy-Rozand et al. (2005b) reported that *E. coli* O157:H7 was detectable even after 42 days of storage, when the milk was initially inoculated with 10, 100 or 1000 CFU/mL. Govaris et al. (2001) reported that *E. coli* O157:H7 could grow during storage at 12 °C in Myzithra, Anthotyros and Manouri whey cheeses within a pH range of 6.3–5.2. D'Amico et al. (2010) have demonstrated the growth and survival of two *E. coli* O157:H7 strains during the manufacture of Gouda and Cheddar cheeses made with raw milk inoculated with 1.3 log CFU/mL of the different *E. coli* O157:H7 strains. These strains remained detectable after enrichment for more than 270 days in both cheeses. It may be concluded that the rate of inactivation of *E. coli* O157:H7 during the ripening time of cheeses could be also depend on the strain and cheese type.

There are few reports in the literature concerning non-O157 STEC, although Montet et al. (2009) found that non-O157 STEC strains survived in Camembert-type cheese during ripening for up to 20 days.

8.2.3. New technologies for the control of STEC in cheese

The application of new technologies may be promising for the control of STEC in cheese. Rodriguez et al. (2005) found that pressurisation at 300 MPa for 10 min at 10 °C, on cheese produced with various bacteriocin starters, reduced *E. coli* O157:H7 to undetectable or levels only detectable by enrichment after 60 days of ripening. After inoculation of *E. coli* O59:H21 and O157:H7 to the curd of a cheese model, high hydrostatic pressure of 400 or 500 MPa decreased the populations of both *E. coli* strains (De Lamo-Castellví et al., 2006).

In general, even with low initial levels of inoculation, *E. coli* O157:H7 (and probably other STEC) can grow during cheese manufacture and survive during cheese ripening at levels high enough to be considered a public health threat (Maher et al., 2001; Montet et al., 2009; Reitsma and Henning, 1996). Therefore, the absence of *E. coli* O157:H7 and other STEC/EHEC from milk destined for cheese manufacture, where there is no inactivation process (such as pasteurisation), is a necessary control measure.

9. Measures for preventing contamination of milk and milk products

As stated in the previous sections, the main reservoir of STEC is the ruminant alimentary tract; bacterial cells are excreted in faeces and primarily contaminate udders, teats and hides (Nastasijevic et al., 2008), and the farm environment where they can persist (Frémaux et al., 2006). Secondly, they can contaminate milking machines and milk lines (Čížek et al., 2008; Murphy et al., 2007) and finally the milk (Anonymous, 2003; Grace et al., 2008) and processed products such as raw milk cheeses (Anonymous, 2003; Baylis, 2009; CDC, 2000; Deschênes et al., 1996; Djuretic et al., 1997; Ercolini et al., 2005; Honish et al., 2005; Ramsaran et al., 1998; Schlesser et al., 2006; Vernozy-Rozand et al., 2005a). Outbreaks can occur from the consumption of these products and also from consumption of recontaminated pasteurised milk or dairy products made from pasteurised milk. There is a comparatively higher risk when milk is pasteurised on the farm (Buvens et al., 2011; Clough et al., 2009).

In order to mitigate against contamination of milk by STEC, several intervention strategies have been explored, either alone or in combination. Such strategies include hygiene practices, prophylaxis, feeding, and consumer information. However, the lack of scientific knowledge on the diversity and the resistance of STEC strains, and the high within- and between-herd heterogeneity of faecal shedding complicates the selection of targeted interventions (Matthews et al., 2006; Frémaux et al., 2006; Fremaux et al., 2007a, 2007b, 2008a, 2008b; Son et al., 2009).

9.1. Interventions at farm level

The primary defence against STEC contamination of milk is milking hygiene, resulting in limiting faecal contamination of the teats, although removal of the natural teat skin microflora should be limited as it contributes to cheese technology and organoleptic quality of some raw

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milk cheeses. Faecal contamination is less probable for sheep and goats than for cows (Beutin et al., 1993; Blanco et al., 2003), as their faeces tend to be more solid.

Where the possibility of contamination from the environment is high, hygienic milking can be insufficient and a number of additional intervention strategies (reviewed by Berry and Wells, 2010) have been suggested in an attempt to decrease the faecal excretion, including detection and isolation of high shedders of STEC (Chase-Topping et al., 2008), prophylaxis/vaccination (Asper et al., 2007; Bretschneider et al., 2007; Dziva et al., 2007; Judge et al., 2004; McNeilly et al., 2008, 2010; Peterson et al., 2007a, 2007b; Sargeant et al., 2007; Smith et al., 2008; Thomson et al., 2009; Thornton et al., 2009), use of probiotics, antimicrobials, sodium chlorate or bacteriophages (Callaway et al., 2004, 2009; Khanna et al., 2008; Loneragan and Brashears, 2005; Sargeant et al., 2007; Rozema et al., 2009) or altering diet (Callaway et al., 2009; Jacob et al., 2009).

To decrease the level and the circulation of STEC on the farm, other interventions have also been attempted as described previously (Anonymous, 2004, 2007a, 2008; CAC, 2004). However, with the current state of scientific knowledge, it is not possible to determine which measure(s) should have priority. Indeed, total eradication of STEC from the farm environment is not possible because of the high prevalence in cattle herds (Collins and Wall, 2004; Hancock et al., 2001).

Where the raw milk is intended for production of raw milk product, selection of farms and involvement and specific skills of producers are recommended and must be considered together with the preservation of the natural microbial population of raw milk.

9.2. Interventions in the milk processing plant and during distribution

There is no specific measure other than pasteurisation that would target STEC reduction during processing (Anonymous, 2004). Therefore, the usual good hygienic practices (GHP) apply, as recommended in guidelines, as well as application of the HACCP principles.

Little work has been carried out on STEC biofilm development within the dairy context, although like other bacteria, *E. coli* O157:H7 is able to attach to and form biofilms on surfaces which are commonly used in food processing environments, such as stainless steel (Dewanti and Wong, 1995; Ryu and Beuchat, 2005). However, this ability seems to be highly strain dependent, and strains which naturally display an enhanced tendency for biofilm development produce curli or fimbriae-like structures (Cookson et al., 2002; Ryu et al., 2004) and the presence of other bacterial populations on a surface may enhance biofilm development of *E. coli* O157 strains (Marouani-Gadri et al., 2009). Biofilm cells are also more resistant to the antimicrobial effects of disinfectants (sanitisers), such as chlorine, than their planktonic counterparts (Ryu and Beuchat, 2005). Studies have indicated that adequate inactivation of biofilm cells of *E. coli* O157:H7 can be achieved when using combination alkaline cleaners coupled with hypochlorite wash solutions (Sharma et al., 2005).

Technological steps, such as micro-filtration or pasteurisation, can decrease the contamination level at processing. These technological measures should be adapted to the type of milk product (e.g. soft vs. hard cheese) after a hazard analysis process. It should be remembered that, as far as raw milk products are concerned, some measures that are efficient in the management of other pathogenic bacteria, such as long term ripening (Schlesser et al., 2006), competitive ecosystems or acidification, may not be useful in reduction of some STEC strains. Further research in this area should be conducted, particularly on non-O157 serogroups. Hygienic practices along the distribution chain and a continuous chill chain are essential in the control of STEC.

9.3. Combination of preventive practices

Combining hurdles to prevent contamination and possible growth of STEC is recommended (Callaway et al., 2009; Vosough Ahmadi et al., 2007). The efficiency of a combination of hurdles at the farm level was studied by Ellis-Iversen et al. (2008). The statistical evidence showed that dry bedding and maintenance of animals in the same groups were the most important measures. Other authors have also attempted to model combined interventions of vaccination, diet, probiotics and water and bedding hygiene (Vosough Ahmadi et al., 2007; Wood et al., 2007).

9.4. Validation, monitoring, verification of control measures

Although there have been outbreaks associated with STEC in raw milk, the occurrence of STEC in raw milk is low (Baylis, 2009; and see Table 3 above). Therefore, end product microbiological analysis for STEC is unlikely to deliver meaningful reductions in associated risk for the consumer (Anonymous, 2003) and the routine monitoring for STEC would not significantly reduce the occurrence of associated cases. Microbiological criteria based on process hygiene, for example based on *E. coli* and/or Enterobacteriaceae, may prove a useful validation, monitoring and verification tool for control measures. A microbiological hygiene criterion based on *E. coli* counts is applied in the European Union for cheeses made from milk or whey that have undergone heat treatment (Anonymous, 2007b). A microbiological criterion is also used for butter and cream made from raw milk or milk that has undergone a lower heat treatment than pasteurisation. This is based on counts of Enterobacteriaceae for milk or whey powder, for ice-cream and frozen dairy desserts, for dried infant formulae and dried dietary foods for special medical purposes intended for infants below 6 months of age, and for dried follow-on formulae.

The use of emerging metrics of microbiological risk management (Anonymous, 2007c), such as a performance objective (PO) could also be useful. A PO is the maximum frequency and/or concentration of a microbiological hazard in a food, at a particular point in the food chain, which should not be exceeded if one is to have confidence that the food safety objective or appropriate level of protection will be maintained. It is expressed in the same way as the microbiological limit of a microbiological criterion (MC) that is in CFU/g. But the PO is a target to be achieved, while the MC is a measurement result that is not equal to the PO, because it has to account for sampling and measurement errors. When the bacterial concentration is too low to be measured, the verification of the PO can only be performed by averaging the results of analyses done on samples analysed sequentially over time (Cerf, 2009). The control of STEC in dairy production can only be achieved by a set of measures along the entire food chain. The optimum combination of these measures remains to be determined and more research is needed to better evaluate the prevalence and numbers along the food chain and the efficacy of control measures, and to develop recognised methods for inexpensive and rapid analyses.

10. Conclusions

While severe cases of bloody diarrhoea or HUS caused by STEC are uncommon, they do affect mostly vulnerable groups such as young children and elderly people, although the outbreak in Germany (ECDC, 2011) in May/June 2011 has highlighted other vulnerable groups. As a result, STEC are perceived as a serious foodborne hazard of increasing concern to authorities and agencies charged with food safety. The most frequent STEC involved in human disease, *E. coli* O157:H7, is commonly known as the “hamburger bug”, yet, sporadic cases and rare outbreaks caused by this and other STEC serogroups, are also due to ingestion of milk or milk products. This review therefore focused on the recent advances in understanding of STEC and their significance in milk and dairy products.

There are many difficulties in assessing occurrence of STEC. Available analytical methods only aim at detecting the serogroups or virulence markers, such as intimin, Shiga toxins, adhesins and

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enterohemolysin associated with strains that caused outbreaks. Again, as seen in the German outbreak, recombination of bacterial DNA leading to new combinations of virulence genes in a new pathogenic type, can pose new challenges in this regard. While standard and validated methods are available and are recommended for use in detection and isolation of *E. coli* O157:H7 from food and animals, there are no standard protocols for their enumeration. For the other STEC serogroups, which are currently considered a potentially significant risk to human health, there are as yet no internationally accepted and validated methods for detection or enumeration. Pragmatic but still rather complicated approaches have been developed. The application of a combination of effective selective and differential plating media and molecular typing methods is needed for routine monitoring and collection of the quantitative data which are essential to better understand the associated human health risks. Availability of such knowledge would help to define more effective processing strategies to reduce contamination of raw food products.

According to EFSA (2007), there are a wide variety of STECs associated with food producing animal populations, for which public health importance remains unclear. Identification of potential virulence factors continues, but understanding of the interactions between virulence factors and host components remains incomplete. It is still not possible to fully define human pathogenic STECs and to know a priori if a given strain is a pathogenic STEC. This can only be done with certainty a posteriori, after isolation from a patient (AFSSA, 2010).

Consequently, only *potentially* pathogenic strains can be detected, leading to too high a number of warnings, or false alarms. If only *stx* genes are sought, the prevalence of potentially pathogenic STEC can appear high. Yet, when isolation of strains and serotyping is carried out, or when the presence of more than one gene is sought, the prevalence of *potentially* pathogenic strains in lactating animal faeces, in milk and in cheeses is most often low, around 1% or less. The possibility of a non-pathogenic strain acquiring pathogenic determinants (like *stx* genes) through recombination remains. As a consequence, a consensus is urgently needed on a scientific definition of pathogenic STECs and the optimal strategy to define the set of pathogenicity determinants that should be sought.

Once milk has been contaminated with STEC, they can subsequently grow in milk products produced from this contaminated milk, if an intervention strategy like pasteurisation is not used. For example, studies have shown that for artificially contaminated soft cheese, *E. coli* O157:H7 is capable of surviving the cheese manufacturing, ripening and storage processes. Many studies have documented the effects of various intervention techniques on *E. coli* O157:H7. Some of these interventions should also be tested on non-O157 serogroups, as it is known that variability among different serogroups is large with regard to growth and survival properties. Further studies are needed to understand the behaviour of STEC (other than O157:H7) in dairy products and understand the impact of matrices and processes on the expression of virulence factors.

At present, milk pasteurisation is generally advised. Regarding the use of raw milk for the production of cheese or other milk products, additional hygiene provisions should be used in the production of such milk (CAC, 2004). Further research is needed to determine the best combination of the available hurdles, viz. appropriate herd management practices, elimination of high-shedders, vaccination, diet and/or probiotics in feed that should be used. In addition, it is necessary to reduce the circulation of strains between farms, and to implement good hygienic practices on the farm. Particularly, not only during raw milk cheese processing, but also for pasteurised dairy products, strict hygiene is essential, and good manufacturing and hygiene practices as well as HACCP principles should be followed to prevent post-pasteurisation contamination. Safety of cheeses is enhanced when production includes a combination of adequate acid production and reduced a_w, such as that found in hard, aged cheeses.

With respect to the prevention of sporadic cases of disease caused by STEC, the question of the relevance of regular monitoring to control STEC in food arises. End-product testing for STEC is not an effective control strategy; therefore, it would be ineffective to set a microbiological criterion because contamination rates in suspect foods are very low. In addition, the presence of STEC is highly improbable so the chance of obtaining isolates from samples in a batch of food is small. The setting of a PO would be more likely to prove useful.

The International Dairy Federation is aware of the risk posed by the occurrence and possible growth of pathogenic strains of STEC in milk and dairy products. It is recognised that effective means for preventing milk contamination at the farm are limited and that pasteurisation ensures the safety of milk and milk products, for the most part. Interventions during raw milk processing are limited and strict hygiene practices at farm level need to be implemented in order to prevent contamination. The number of cases of severe disease caused by STEC in dairy products has remained quite low, probably thanks to the compliance with good hygienic practices at the farm level.

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References

Adesiyun, A.A., Stoute, S., David, B., 2007. Pre-processed bovine milk quality in Trinidad: prevalence and characteristics of bacterial pathogens and occurrence of antimicrobial residues in milk from collection centers. Food Control 18, 312–320.

AFSSA (Agence Française de Sécurité Sanitaire des Aliments), 2010. Avis de l'Agence française de sécurité sanitaire des aliments relatif à la pertinence d'une révision de la définition des STEC pathogènes, précisée par l'avis Afssa du 15 juillet 2008, 27 mai 2010, pp. 1–19 (Afssa saisine no 2010-SA-0122. Maisons-Alfort, France. Available at http://www.afssa.fr/Documents/MIC2010sa0031.pdf. Last accessed 17th October 2011).

Allerberger, F., Wagner, M., Schweiger, P., Rammer, H.P., Resch, A., Dierich, M.P., Friedrich, A.W., Karch, H., 2001. Infections à *Escherichia coli* O157 associées au lait non pasteurisé. Eurosurveillance 6 (article 379, 2001-10-01).

Allerberger, F., Friedrich, A.W., Grif, K., Dierich, M.P., Dornbusch, H.J., Mache, C.J., Nachbaur, E., Freilinger, M., Rieck, P., Wagner, M., 2003. Hemolytic–uremic syndrome associated with enterohemorrhagic *Escherichia coli* O26:H infection and consumption of unpasteurized cow's milk. International Journal of Infectious Diseases 7, 42–45.

Almeida, G., Figueiro, A., Rola, M., Barros, R.M., Gibbs, P., Hogg, T., Teixeira, P., 2007. Microbiological characterization of randomly selected Portuguese raw milk cheeses with reference to food safety. Journal of Food Protection 70, 1710–1716.

Ammon, A., 1997. Surveillance of enterohaemorrhagic *E. coli* (EHEC) infections and haemolytic uraemic syndrome (HUS) in Europe. Eurosurveillance 2, 91–95 (article 133, 1997-12-01).

Anonymous, 1999. Outbreak of vero cytotoxin-producing *E. coli* O157 infection in North Cumbria. CDR Weekly 9, 95–98 (http://www.hpa.org.uk/cdr/archives/1999/cdr1199.pdf. Last accessed 17th October 2011).

Anonymous, 2003. Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on Verotoxigenic *E. coli* (VTEC) in Foodstuffs. European Commission — Health & Consumer Protection Directorate-General, Brussels, Belgium.

Anonymous, 2004. Code of good hygienic practices for milking with automatic milking systems. Bulletin of the International Dairy Federation 386, 43–49 (2004).

Anonymous, 2007a. Good dairy farming practices related to primary production of milk and farm management. Bulletin of the International Dairy Federation 418, 3–42 (2007).

Anonymous, 2007b. Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Official Journal of the European Union L322, 12–29.

Anonymous, 2007c. Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM) — CAC/GL 63-2007. Codex Alimentarius Commission, Rome, Italy.

Anonymous, 2008. Guide to Good Animal Welfare in Dairy Production. International Dairy Federation, Brussels, Belgium.

Ansay, S.E., Kaspar, C.W., 1997. Survey of retail cheeses, dairy processing environments and raw milk for *Escherichia coli* O157:H7. Letters in Applied Microbiology 25, 131–134.

Arimi, S.M., Koroti, E., Kang'ethe, E.K., Omore, A.O., McDermott, J.J., 2005. Risk of infection with *Brucella abortus* and *Escherichia coli* O157:H7 associated with marketing of unpasteurized milk in Kenya. Acta Tropica 96, 1–8.

Asper, D.J., Sekirov, I., Finlay, B.B., Rogan, D., Potter, A.A., 2007. Cross reactivity of enterohemorrhagic *Escherichia coli* O157:H7-specific sera with non-O157 serotypes. Vaccine 25, 8262–8269.

Auvray, F., Lecureuil, C., Tache, J., Leclerc, V., Deperrois, V., Lombard, B., 2007. Detection, isolation and characterization of Shiga toxin-producing *Escherichia coli* in retail-minced beef using PCR-based techniques, immunoassays and colony hybridization. Letters in Applied Microbiology 45, 646–651.

Auvray, F., Lecureuil, C., Dilasser, F., Tache, J., Derzelle, S., 2009. Development of a real-time PCR assay with an internal amplification control for the screening of Shiga toxin-producing *Escherichia coli* in foods. Letters in Applied Microbiology 48, 554–559.

Avery, S.M., Moore, A., Hutchinson, M.L., 2004. Fate of *Escherichia coli* originating from livestock faeces deposited directly onto pasture. Letters in Applied Microbiology 38, 355–359.

Ballmer, K., Korczak, B.M., Kuhnert, P., Slickers, P., Ehricht, R., Hachler, H., 2007. Fast DNA serotyping of *Escherichia coli* by use of an oligonucleotide microarray. Journal of Clinical Microbiology 45, 370–379.

Bastian, S.N., Sivelä, S.K., 2000. *Escherichia coli* O157:H7: aspects of concern to the dairy industry. Bulletin of the International Dairy Federation 357, 57–62 (2000).

Bastian, S.N., Carle, I., Grimont, F., 1998. Comparison of 14 PCR systems for the detection and subtyping of stx genes in Shiga-toxin-producing *Escherichia coli*. Research in Microbiology 149, 457–472.

Baylis, C.L., 2008. Growth of pure cultures of verocytotoxin-producing *Escherichia coli* in a range of enrichment media. Journal of Applied Microbiology 105, 1259–1265.

Baylis, C.L., 2009. Raw milk and raw milk cheeses as vehicles for infection by verocytotoxin-producing *Escherichia coli*. International Journal of Dairy Technology 62, 293–307.

Becker, A.L., 2005. Alberta *E. coli* outbreak tied to milk shakes. Press release CIDRAP. 2005-05-13.

Belanger, S.D., Boissinot, M., Menard, C., Picard, F.J., Bergeron, M.G., 2002. Rapid detection of Shiga toxin-producing bacteria in feces by multiplex PCR with molecular beacons on the smart cycler. Journal of Clinical Microbiology 40, 1436–1440.

Berry, E.D., Wells, J.E., 2010. *Escherichia coli* O157:H7: recent advances in research on occurrence, transmission, and control in cattle and the production environment. Advances in Food and Nutrition Research 60, 67–117 (Chapter 4).

Besser, R.E., Lett, S.M., Weber, J.T., Doyle, M.P., Barrett, T.J., Wells, J.G., Griffin, P.M., 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. Journal of the American Medical Association 269, 2217–2220.

Bettelheim, K.A., 2007. The non-O157 Shiga-toxigenic (verocytotoxigenic) *Escherichia coli*: under-rated pathogens. Critical Reviews in Microbiology 33, 67–87.

Betts, C.D., 2000. Controlling *E. coli* O157:H7. Nutrition and Food Science 30, 183–186.

Beutin, L., Montenegro, M.A., Orskov, I., Orskov, F., Prada, J., Zimmermann, S., Stephan, R., 1989. Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. Journal of Clinical Microbiology 27, 2559–2564.

Beutin, L., Geier, D., Steinrück, H., Zimmermann, S., Scheutz, F., 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. Journal of Clinical Microbiology 31, 2483–2488.

Beutin, L., Miko, A., Krause, G., Pries, K., Haby, S., Steege, K., Albrecht, N., 2007. Identification of human-pathogenic strains of Shiga toxin-producing *Escherichia coli* from food by a combination of serotyping and molecular typing of Shiga toxin genes. Applied and Environmental Microbiology 73, 4769–4775.

Beutin, L., Jahn, S., Fach, P., 2009. Evaluation of the 'GeneDisc' real-time PCR system for detection of enterohaemorrhagic *Escherichia coli* (EHEC) O26, O103, O111, O145 and O157 strains according to their virulence markers and their O- and H-antigen-associated genes. Journal of Applied Microbiology 106, 1122–1132.

Bielaszewska, M., Janda, J., Bláhová, K., Minaríková, H., Jíková, E., Karmali, M.A., Laubová, J., Šikulová, J., Preston, M.A., Khakhria, R., Karch, H., Klazarová, H., Nyc, O., 1997. Human *Escherichia coli* O157:H7 infection associated with the consumption of unpasteurized goat's milk. Epidemiology and Infection 119, 299–305.

Bielaszewska, M., Friedrich, A.W., Aldick, T., Schurk-Bulgrin, R., Karch, H., 2006. Shiga toxin activatable by intestinal mucus in *Escherichia coli* isolated from humans: predictor for a severe clinical outcome. Clinical Infectious Diseases 43, 1160–1167.

Bielaszewska, M., Mellmann, A., Zhang, W., Köck, R., Fruth, A., Bauwens, A., Peters, G., Karch, H., 2011. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. The Lancet Infectious Diseases 11, 671–676.

Blanco, M., Blanco, J.E., Mora, A., Rey, J., Alonso, J.M., Hermoso, M., Hermoso, J., Alonso, M.P., Dahbi, G., Gonzalez, E.A., Bernardez, M.I., Blanco, J., 2003. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from healthy sheep in Spain. Journal of Clinical Microbiology 41, 1351–1356.

Bockemühl, J., Karch, H., 1996. Zur aktuellen Bedeutung der enterohämorrhagischen. *Escherichia coli* (EHEC) in Deutschland (1994–1995). Bundesgesundheitsblatt 39, 290–296.

Bockemühl, J., Karch, H., Rüssmann, H., Aleksic, S., Wiss, R., Emmrich, P., 1990. Shiga-like toxin (verotoxin)-produzierende *Escherichia coli* O22:H8: Übertragung durch unpasteurisierte Milch mit nachfolgender Erkrankung an hämolytisch-urämischen Syndrom. Bundesgesundheitsblatt 33, 3–6.

Bonnet, R., Souweine, B., Gauthier, G., Rich, C., Livrelli, V., Sirot, J., Joly, B., Forestier, C., 1998. Non-O157:H7 Stx2-producing *Escherichia coli* strains associated with sporadic cases of hemolytic–uremic syndrome in adults. Journal of Clinical Microbiology 36, 1777–1780.

Botteldoorn, N., Heyndrickx, M., Rijpens, N., Herman, L., 2003. Detection and characterization of verotoxigenic *Escherichia coli* by a VTEC/EHEC multiplex PCR in porcine faeces and pig carcass swabs. Research in Microbiology 154, 97–104.

Bretschneider, G., Berberov, E.M., Moxley, R.A., 2007. Isotype-specific antibody responses against *Escherichia coli* O157:H7 locus of enterocyte effacement proteins in adult beef cattle following experimental infection. Veterinary Immunology and Immunopathology 118, 229–238.

Brunder, W., Schmidt, H., Frosch, M., Karch, H., 1999. The large plasmids of Shiga-toxin-producing *Escherichia coli* (STEC) are highly variable genetic elements. Microbiology 145, 1005–1014.

Brzuszkiewicz, E., Thurmer, A., Schuldes, J., Leimbach, A., Liesegang, H., Meyer, F.D., Boelter, J., Petersen, H., Gottschalk, G., Daniel, R., 2011. Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: Entero-Aggregative-Haemorrhagic *Escherichia coli* (EAHEC). Archives of Microbiology 193, 883–891.

Bugarel, M., Beutin, L., Fach, P., 2010a. Low-density macroarray targeting non-locus of enterocyte effacement effectors (nle genes) and major virulence factors of Shiga toxin-producing *Escherichia coli* (STEC): a new approach for molecular risk assessment of STEC isolates. Applied and Environmental Microbiology 76, 203–211.

Bugarel, M., Beutin, L., Martin, A., Gill, A., Fach, P., 2010b. Micro-array for the identification of Shiga toxin-producing *Escherichia coli* (STEC) seropathotypes associated with hemorrhagic colitis and hemolytic uremic syndrome in humans. International Journal of Food Microbiology 142, 318–329.

Buglino, D., Smith-Sayer, K., Fortino, S., Van Meter, J., Frykman, E., Crawford-Miksza, L., Myers, C., Himathongkham, S., Palumbo, M., Atwell, J., Chang, T., Csuti, D., Fontanoz, S., Gerbremichael, Y., Glover, J., O'Connell, J., Sun, B., Wheeler, C., Zhao, Y., Lynch, M., 2008. *Escherichia coli* O157:H7 infections in children associated with raw milk and raw colostrum from cows — California, 2006. Morbidity and Mortality Weekly Report 57, 625–628.

Buvens, G., Possé, B., De Schrijver, K., De Zutter, L., Lauwers, S., Pierard, D., 2011. Virulence profiling and quantification of verocytotoxin-producing *Escherichia coli* O145:H28 and O26:H11 isolated during an ice cream-related haemolytic uremic syndrome outbreak. Foodborne Pathogens and Disease 8, 421–426.

Byrne, C.M., Erol, I., Call, J.E., Kaspar, C.W., Buege, D.R., Hiemke, C.J., Fedorka-Cray, P.J., Benson, A.K., Wallace, F.M., Luchansky, J.B., 2003. Characterization of *Escherichia coli* O157:H7 from downer and healthy dairy cattle in the Upper Midwest Region of the United States. Applied and Environmental Microbiology 69, 4683–4688.

CAC (Codex Alimentarius Commission), 2004. Code of Hygienic Practice for Milk and Milk Products. Codex Alimentarius Commission, Rome, Italy.

Callaway, T.R., Anderson, R.C., Edrington, T.S., Genovese, K.J., Harvey, R.B., Poole, T.L., Nisbet, D.J., 2004. Recent pre-harvest supplementation strategies to reduce carriage and shedding of zoonotic enteric bacterial pathogens in food animals. Animal Health Research Reviews 5, 35–47.

Callaway, T.R., Carr, M.A., Edrington, T.S., Anderson, R.C., Nisbet, D.J., 2009. Diet, *Escherichia coli* O157:H7, and cattle: a review after 10 years. Current Issues in Molecular Biology 11, 67–80.

Caro, I., Garcia-Armesto, M.R., 2007. Occurrence of Shiga toxin-producing *Escherichia coli* in a Spanish raw ewe's milk cheese. International Journal of Food Microbiology 116, 410–413.

Casenave, C., Desenclos, J.C., Maillot, E., Benoît, S., Deschenes, G., Nivet, H., Grimont, F., Baron, S., Mariani, P., Grimont, P.A.D., 1993. Éclosion de syndrome hémolytique et urémique dans une commune rurale du Cher. Bulletin Epidémiologique Hebdomadaire 48, 222–224.

Castanie-Cornet, M.P., Penfound, T.A., Smith, D., Elliott, J.F., Foster, J.W., 1999. Control of acid resistance in *Escherichia coli*. Journal of Bacteriology 181, 3525–3535.

CDC (Centers for Diseases Control and Prevention), 1995a. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami—Washington and California, 1994. Morbidity and Mortality Weekly Report 44, 157–160.

CDC (Centers for Diseases Control and Prevention), 1995b. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21 — Helena, Montana, 1994. Morbidity and Mortality Weekly Report 44, 501–503.

CDC (Centers for Diseases Control and Prevention), 1997. *Escherichia coli* O157:H7 infections associated with eating a nationally distributed commercial brand of frozen ground beef patties and burgers—Colorado, 1997. Morbidity and Mortality Weekly Report 46, 777–778.

CDC (Centers for Diseases Control and Prevention), 2000. Outbreak of *Escherichia coli* O157:H7 infection associated with eating fresh cheese curds—Wisconsin, June 1998. Morbidity and Mortality Weekly Report 49, 911–913.

CDC (Centers for Diseases Control and Prevention), 2006. Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach—United States, September 2006. Morbidity and Mortality Weekly Report 55, 1045–1046.

CDC (Centers for Diseases Control and Prevention), 2007a. *Escherichia coli* O157:H7 infection associated with drinking raw milk — Washington and Oregon, November–December 2005. Morbidity and Mortality Weekly Report 56, 165–167.

CDC (Centers for Diseases Control and Prevention), 2007b. Foodborne Outbreak Online Database, 2007 July, Colorado, *E. coli* Shiga Toxin Producing O121, O26, O84, Prison Jail. American Cheese Pasteurized. (http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx. Last accessed December 24th 2011).

CDC (Centers for Diseases Control and Prevention), 2008. *Escherichia coli* 0157:H7 infections in children associated with raw milk and raw colostrum from cows — California, 2006. Morbidity and Mortality Weekly Report 57, 625–628.

CDC (Centers for Diseases Control and Prevention), 2010. Investigation Update: Multistate Outbreak of *E. coli* O157:H7 Infections Associated with Cheese. (2010-11-24).

CDSC (Communicable Disease Surveillance Centre), 1998a. Cases of *Escherichia coli* O157 infection associated with unpasteurised cream in England. Eurosurveillance 2, 1135 (1998-10-29).

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CDSC (Communicable Disease Surveillance Centre), 1998b. Outbreak of vero cytotoxin producing *Escherichia coli* O157 infection in Dorset. CDR Weekly: Communicable Disease Report 8 (183), 186.
Cerf, O., 2009. Modern Concepts of Microbiological Risk Management, from a "Hazard Focused" to a "Risk Focused" Approach, IDF World Dairy Summit "United Dairy World". (Berlin, Germany, 20–24 September 2009).
Chao, G., Zhou, X., Jiao, X., Qian, X., Xu, L., 2007. Prevalence and antimicrobial resistance of foodborne pathogens isolated from food products in China. Foodborne Pathogens and Disease 4, 277–284.
Chase-Topping, M., Gally, D., Low, C., Matthews, L., Woolhouse, M., 2008. Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. Nature Reviews Microbiology 6, 904–912.
Civera, T., Decastelli, L., Martorana, M., Dalmasso, A., Nucera, D., Bottero, M.T., 2007. Survey on the presence of enterovirulent *Escherichia coli*, including *E. coli* O157, in cheese manufactured in the Italian Alpine Region. International Journal of Food Science 19, 229–238.
Čížek, A., Dolejská, M., Novotná, R., Haas, D., Vyskočil, M., 2008. Survey of Shiga toxigenic *Escherichia coli* O157 and drug-resistant coliform bacteria from in-line milk filters on dairy farms in the Czech Republic. Journal of Applied Microbiology 104, 852–860.
Clark, A., Morton, S., Wright, P., Corkish, J., Bolton, F.J., Russell, J., 1997. A community outbreak of vero-cytotoxin producing *Escherichia coli* O157 infection linked to a small farm dairy. Communicable Disease Report. CDR Review 7, R206–R211.
Clough, H.E., Clancy, D., French, N.P., 2009. Quantifying exposure to vero-cytotoxigenic *Escherichia coli* O157 in milk sold as pasteurized: a model-based approach. International Journal of Food Microbiology 131, 95–105.
Cobbaut, K., Berkvens, D., Houf, K., De Deken, R., De Zutter, L., 2009. *Escherichia coli* O157 prevalence in different cattle farm types and identification of potential risk factors. Journal of Food Protection 72, 1848–1853.
Cobbold, R., Desmarchelier, P., 2001. Characterisation and clonal relationships of Shiga-toxigenic *Escherichia coli* (STEC) isolated from Australian dairy cattle. Veterinary Microbiology 79, 323–335.
Cobbold, R., Desmarchelier, P., 2002. Horizontal transmission of Shiga toxin-producing *Escherichia coli* within groups of dairy calves. Applied and Environmental Microbiology 68, 4148–4152.
Cobbold, R.N., Davis, M.A., Rice, D.H., Szymanski, M., Tarr, P.I., Besser, T.E., Hancock, D.D., 2008. Associations between bovine, human, and raw milk, and beef isolates of non-O157 Shiga toxigenic *Escherichia coli* within a restricted geographic area of the United States. Journal of Food Protection 71, 1023–1027.
Cody, S., Glynn, M., Farrar, J., Cairns, K., Griffin, P., Kobayashi, J., Fyfe, M., Hoffman, R., King, A., Lewis, J., Swaminathan, B., Bryant, R., Vugia, D., 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. Annals of Internal Medicine 130, 202–209.
Coia, J.E., Johnston, Y., Steers, N.J., Hanson, M.F., 2001. A survey of the prevalence of *Escherichia coli* O157 in raw meats, raw cow's milk acid raw-milk cheeses in south-east Scotland. International Journal of Food Microbiology 66, 63–69.
Collins, J.D., Wall, P.G., 2004. Food safety and animal production systems: controlling zoonoses at farm level. Revue scientifique et technique de l'Office international des épizooties, 23, pp. 685–700.
Conedera, G., Dalvit, P., Martini, M., Galiero, G., Gramaglia, M., Goffredo, E., Loffredo, G., Morabito, S., Ottaviani, D., Paterlini, F., Pezzotti, G., Pisanu, M., Semprini, P., Caprioli, A., 2004. Verocytotoxin-producing *Escherichia coli* O157 in minced beef and dairy products in Italy. International Journal of Food Microbiology 96, 67–73.
Cookson, A.L., Cooley, W.A., Woodward, M.J., 2002. The role of type 1 and curli fimbriae of Shiga toxin-producing *Escherichia coli* in adherence to abiotic surfaces. International Journal of Medical Microbiology 292, 195–205.
Cookson, A.L., Taylor, S.C.S., Benett, J., Thomson-Carter, F., Attwood, G.T., 2006. Serotypes and analysis of distribution of Shiga toxin-producing *Escherichia coli* from cattle and sheep in the lower North Island, New Zealand. New Zealand Veterinary Journal 54, 78–84.
Cornick, N.A., Helgerson, A.F., Mai, V., Ritchie, J.M., Acheson, D.W., 2006. In vivo transduction of an Stx-encoding phage in ruminants. Applied and Environmental Microbiology 72, 5086–5088.
Cortés, C., De la Fuente, R., Blanco, J., Blanco, M., Blanco, J.E., Dhabi, G., Mora, A., Justel, P., Contreras, A., Sánchez, A., Corrales, J.C., Orden, J.A., 2005. Serotypes, virulence genes and intimin types of verotoxin-producing *Escherichia coli* and enteropathogenic *E. coli* isolated from healthy dairy goats in Spain. Veterinary Microbiology 110, 67–76.
Curnow, J., 1999. *Escherichia coli* O157 outbreak in Scotland linked to unpasteurised goat's milk. Eurosurveillance 3 (article 1387).
D'Amico, D.J., Groves, E., Donnelly, C.W., 2008. Low incidence of foodborne pathogens of concern in raw milk utilized for farmstead cheese production. Journal of Food Protection 71, 1580–1589.
D'Amico, D.J., Druart, M.J., Donnelly, C.W., 2010. Behavior of *Escherichia coli* O157:H7 during the manufacture and aging of Gouda and stirred-curd Cheddar cheeses manufactured from raw milk. Journal of Food Protection 73, 2217–2224.
D'Aoust, J.Y., Park, C.E., Szabo, R.A., Todd, E.C.D., 1988. Thermal inactivation of *Campylobacter* species, *Yersinia enterocolitica*, and hemorrhagic *Escherichia coli* O157:H7 in fluid milk. Journal of Dairy Science 71, 3226–3230.
De Lamo-Castellví, S., Capellas, M., Roig-Sagués, A.X., López-Pedemonte, T., Hernández-Herrero, M.M., Guamis, B., 2006. Fate of *Escherichia coli* strains inoculated in model cheese elaborated with or without starter and treated by high hydrostatic pressure. Journal of Food Protection 69, 2856–2864.
De Reu, K., Debeuckelaere, W., Botteldoorn, N., De Block, J., Herman, L., 2002. Hygienic parameters, toxins and pathogen occurrence in raw milk cheeses. Journal of Food Safety 22, 183–196.
De Reu, K., Grijspeerdt, K., Herman, L., 2004. A Belgian survey of hygiene indicator bacteria and pathogenic bacteria in raw milk and direct marketing of raw milk farm products. Journal of Food Safety 24, 17–36.
De Schrijver, K., Buvens, G., Possé, B., Van den Branden, D., Oosterlynck, C., De Zutter, L., Eilers, K., Piérard, D., Dierick, K., Van Damme-Lombaerts, R., Lauwers, C., Jakobs, R., 2008. Outbreak of verocytotoxin-producing *E. coli* O145 and O26 infections associated with consumption of ice cream produced at a farm, Belgium, 2007. Eurosurveillance 13 (article 8041, 2008-02-14).
De Vuyst, L., Vandamme, E.J., 1992. Influence of the carbon source on nisin production in *Lactococcus lactis* subsp. *lactis* batch fermentations. Journal of General Microbiology 138, 571–578.
Dean, P., Kenny, B., 2009. The effector repertoire of enteropathogenic *E. coli*: ganging up on the host cell. Current Opinion in Microbiology 12, 101–109.
Decludt, B., 1995. Cas groupés de syndromes hémolytiques et urémiques dans le département de l'Ardèche en 1994. Institut de Veille Sanitaire, France.
Delignette-Muller, M.-L., Cornu, M., the AFSSA STEC Study Group, 2008. Quantitative risk assessment for *Escherichia coli* O157:H7 in frozen ground beef patties consumed by young children in French households. International Journal of Food Microbiology 128, 158–164.
Derzelle, S., Grine, A., Madic, J., Peytavin de Garam, C., Vingadassalon, N., Dilasser, F., Jamet, E., Auvray, F., 2011. A quantitative PCR assay for the detection and quantification of Shiga toxin-producing *Escherichia coli* (STEC) in minced beef and dairy products. International Journal of Food Microbiology 151, 44–51.
Deschênes, G., Casenave, C., Grimont, F., Desenclos, J.C., Benoit, S., Collin, M., Baron, S., Mariani, P., Grimont, P.A., Nivet, H., 1996. Cluster of cases of haemolytic uraemic syndrome due to unpasteurised cheese. Pediatric Nephrology 10, 203–205.
Dewanti, R., Wong, A.C.L., 1995. Influence of culture conditions on biofilm formation by *Escherichia coli* O157:H7. International Journal of Food Microbiology 26, 147–164.
Dineen, S.S., Takeuchi, K., Soudah, J.E., Boor, K.J., 1998. Persistence of *Escherichia coli* O157:H7 in dairy fermentation systems. Journal of Food Protection 61, 1602–1608.
Dinu, L.-D., Bach, S., 2011. Induction of viable but non-culturable *Escherichia coli* O157:H7 on the phyllosphere of lettuce: a food safety risk factor. Applied and Environmental Microbiology 77, 8295–8302.
Djuretic, T., Wall, P.G., Nichols, G., 1997. General outbreaks of infectious intestinal disease associated with milk and dairy products in England and Wales: 1992 to 1996. Communicable Disease Report CDR Rev., 7, pp. R41–R45.
Dontorou, C., Papadopoulou, C., Filioussis, G., Economou, V., Apostolou, I., Zakkas, G., Salamoura, A., Kansouzidou, A., Levidiotou, S., 2003. Isolation of *Escherichia coli* O157:H7 from foods in Greece. International Journal of Food Microbiology 82, 273–279.
Doyle, M.P., Schoeni, J.L., 1984. Survival and growth-characteristics of *Escherichia coli* associated with hemorrhagic colitis. Applied and Environmental Microbiology 48, 855–856.
Dziva, F., Vlisidou, I., Crepin, V.F., Wallis, T.S., Frankel, G., Stevens, M.P., 2007. Vaccination of calves with EspA, a key colonisation factor of *Escherichia coli* O157:H7, induces antigen-specific humoral responses but does not confer protection against intestinal colonisation. Veterinary Microbiology 123, 254–261.
ECDC (European Centre for Disease Prevention and Control), EFSA (European Food Safety Authority), 2011. Shiga Toxin/Verotoxin-producing *Escherichia coli* in Humans, Food and Animals in the EU/EEA, with Special Reference to the German Outbreak Strain STEC O104. ECDC, Stockholm.
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 identification of human pathogenic VTEC. The EFSA Journal 579, 1–61.
EFSA (European Food Safety Authority), 2009a. Technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food (VTEC surveys on animals and food) on request of EFSA. The EFSA Journal 7, 1366.
EFSA (European Food Safety Authority), 2009b. The community summary report on food-borne outbreaks in the European Union in 2007. The EFSA Journal 271.
EFSA (European Food Safety Authority), 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 8, 1496.
EFSA (European Food Safety Authority), 2011. Shiga toxin-producing *E. coli* (STEC) O104:H4 outbreaks in Europe: taking stock. The EFSA Journal 9, 2390.
EFSA (European Food Safety Authority), ECDC (European Centre for Disease Prevention and Control), 2011. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. The EFSA Journal 3 (2090), 1–378.
Ellis-Iversen, J., Smith, R.P., Van Winden, S., Paiba, G.A., Watson, E., Snow, L.C., Cook, A.J.C., 2008. Farm practices to control *E. coli* O157 in young cattle—a randomised controlled trial. Veterinary Research 39, 1–12.
Eppinger, M., Mammel, M.K., LeClerc, J.E., Ravel, J., Cebula, T.A., 2011. Genome signatures of *Escherichia coli* O157:H7 isolates from the bovine host reservoir. Applied and Environmental Microbiology 77, 2916–2925.
Ercolini, D., Fusco, V., Blaiotta, G., Sarghini, F., Coppola, S., 2005. Response of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus* to the thermal stress occurring in model manufactures of grana padano cheese. Journal of Dairy Science 88, 3818–3825.
Erickson, M.C., Doyle, M.P., 2007. Food as a vehicle for transmission of Shiga toxin-producing *Escherichia coli*. Journal of Food Protection 70, 2426–2449.
Erilsson, E., Aspan, A., Gunnarsson, A., Vagsholm, I., 2005. Prevalence of verotoxin-producing *Escherichia coli* (VTEC) O157 in Swedish dairy herds. Epidemiology and Infection 133, 349–358.
Espié, E., Vaillant, V., Mariani-Kurkdjian, P., Grimont, F., Martin-Schaller, R., De Valk, H., Vernozy-Rozand, C., 2006. *Escherichia coli* O157 outbreak associated with fresh unpasteurized goats' cheese. Epidemiology and Infection 134, 143–146.

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Ethelberg, S., Smith, B., Torpdahl, M., Lisby, M., Boel, J., Jensen, T., Mølbak, K., 2007. An outbreak of verocytotoxin-producing *Escherichia coli* O26:H11 caused by beef sausage, Denmark 2007. Eurosurveillance 12 (article 3208, 2007-05-31).

Ethelberg, S., Smith, B., Torpdahl, M., Lisby, M., Boel, J., Jensen, T., Nielsen, E., Mølbak, K., 2009. Outbreak of non-O157 Shiga toxin-producing *Escherichia coli* infection from consumption of beef sausage. Clinical Infectious Diseases 48, e78–e81.

Fach, P., Perelle, S., Dilasser, F., Grout, J., 2001. Comparison between a PCR-ELISA test and the vero cell assay for detecting Shiga toxin-producing *Escherichia coli* in dairy products and characterization of virulence traits of the isolated strains. Journal of Applied Microbiology 90, 809–818.

Faith, N.G., Shere, J.A., Brosch, R., Arnold, K.W., Ansay, S.E., Lee, M.S., Luchansky, J.B., Kaspar, C.W., 1996. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. Applied and Environmental Microbiology 62, 1519–1525.

FAO (Food and Agriculture Organization), 2011. Food Outlook, Global Market Analysis, pp. 43–44 (http://www.fao.org/giews/english/fo/index.htm. Last accessed 17th October 2011).

Fedio, W.M., Jinneman, K.C., Yoshitomi, K.J., Zapata, R., Wendakoon, C.N., Browning, P., Weagant, S.D., 2011. Detection of *E. coli* O157:H7 in raw ground beef by Pathatrix immunomagnetic-separation, real-time PCR and cultural methods. International Journal of Food Microbiology 148, 87–92.

Feng, P., Weagant, S.D., Monday, S.R., 2001. Genetic analysis for virulence factors in *Escherichia coli* O104:H21 that was implicated in an outbreak of hemorrhagic colitis. Journal of Clinical Microbiology 39, 24–28.

Food Poison Journal, 2009-12-02, presented by Marler Clark. http://www.foodpoisonjournal.com/foodborne-illness-outbreaks/raw-milk-e-coli-strikes-again-recent-illnesses-are-reminder-of-risks-from-drinking-raw-milk/. Last accessed 19th April 2012.

Foschino, R., Invernizzi, A., Barucco, A., Stradiotto, K., 2002. Microbial composition, including the incidence of pathogens, of goat milk from the Bergamo region of Italy during a lactation year. The Journal of Dairy Research 69, 213–225.

Foster, J.W., 2004. *Escherichia coli* acid resistance: tales of an amateur acidophile. Nature Reviews Microbiology 2, 898–907.

Fox, P.F., McSweeney, P.L.H., 2004. Cheese: an overview. In: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), Cheese: Chemistry. : Physics and Microbiology, 1. Elsevier Academic Press, London, pp. 1–18.

Fratamico, P.M., DebRoy, C., Miyamoto, T., Liu, Y., 2009. PCR detection of enterohemorrhagic *Escherichia coli* O145 in food by targeting genes in the *E. coli* O145 O-antigen gene cluster and the Shiga toxin 1 and Shiga toxin 2 genes. Foodborne Pathogens and Disease 6, 605–611.

Fratamico, P.M., Bagi, L.K., Cray, W.C., Narang, N., Yan, X., Medina, M., Liu, Y., 2011. Detection by multiplex real-time polymerase chain reaction assays and isolation of Shiga toxin-producing *Escherichia coli* serogroups O26, O45, O103, O111, O121, and O145 in ground beef. Foodborne Pathogens and Disease 8, 601–607.

Frémaux, B., Raynaud, S., Beutin, L., Vernozy-Rozand, C., 2006. Dissemination and persistence of Shiga toxin-producing *Escherichia coli* (STEC) strains on French dairy farms. Veterinary Microbiology 117, 180–191.

Fremaux, B., Delignette-Muller, M.L., Prigent-Combaret, C., Gleizal, A., Vernozy-Rozand, C., 2007a. Growth and survival of non-O157:H7 Shiga-toxin-producing *Escherichia coli* in cow manure. Journal of Applied Microbiology 102, 89–99.

Fremaux, B., Prigent-Combaret, C., Delignette-Muller, M.L., Dothal, M., Vernozy-Rozand, C., 2007b. Persistence of Shiga toxin-producing *Escherichia coli* O26 in cow slurry. Letters in Applied Microbiology 45, 55–61.

Fremaux, B., Prigent-Combaret, C., Delignette-Muller, M.L., Mallen, B., Dothal, M., Gleizal, A., Vernozy-Rozand, C., 2008a. Persistence of Shiga toxin-producing *Escherichia coli* O26 in various manure-amended soil types. Journal of Applied Microbiology 104, 296–304.

Fremaux, B., Prigent-Combaret, C., Vernozy-Rozand, C., 2008b. Long-term survival of Shiga toxin-producing *Escherichia coli* in cattle effluents and environment: an updated review. Veterinary Microbiology 25, 1–18.

Friedrich, A.W., Bielaszewska, M., Zhang, W.L., Pulz, M., Kuczius, T., Ammon, A., Karch, H., 2002. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. Journal of Infectious Diseases 185, 74–84.

Galland, J.C., Hyatt, D.R., Crupper, S.S., Acheson, D.W., 2001. Prevalence, antibiotic susceptibility and diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. Applied and Environmental Microbiology 67, 1619–1627.

Gálvez, A., Abriouel, H., López, R.L., Ben Omar, N., 2007. Bacteriocin-based strategies for food biopreservation. International Journal of Food Microbiology 120, 51–70.

Garber, L.P., Wells, S., Schroeder-Tucker, L., Ferris, K., 1999. Factors associated with fecal shedding of verotoxin-producing *Escherichia coli* O157 on dairy farms. Journal of Food Protection 62, 307–312.

Garmendia, J., Frankel, G., Crepin, V.F., 2005. Enteropathogenic and enterohemorrhagic *Escherichia coli* infections: translocation, translocation, translocation. Infection and Immunity 73, 2573–2585.

Geue, L., Klare, S., Schnick, C., Mintel, B., Meyer, K., Conraths, F.J., 2009. Analysis of the clonal relationship of serotype O26:H11 enterohemorrhagic *Escherichia coli* isolates from cattle. Applied and Environmental Microbiology 75, 6947–6953.

Glass, K.A., Loeffelholz, J.M., Ford, J.P., Doyle, M.P., 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Applied and Environmental Microbiology 58, 2513–2516.

Govaris, A., Koidis, P., Papatheodorou, K., 2001. The behaviour of *Escherichia coli* O157:H7 in whey cheeses Myzithra, Anthotyros and Manouri during storage at 2 °C and 12 °C. Food Microbiology 18, 565–570.

Govaris, A., Papageorgiou, D.K., Papatheodorou, K., 2002. Behavior of *Escherichia coli* O157:H7 during the manufacture and ripening of feta and telemes cheeses. Journal of Food Protection 65, 609–615.

Grace, D., Omore, A., Randolph, T., Kang'ethe, E., Nasinyama, G., Mohammed, H., 2008. Risk assessment for *Escherichia coli* O157:H7 in marketed unpasteurized milk in selected East African countries. Journal of Food Protection 71, 257–263.

Grzadkowska, D., Griffiths, M.W., 2001. Cryotolerance of *Escherichia coli* O157:H7 in laboratory media and food. Journal of Food Science 66, 1169–1173.

Gyles, C., 2007. Shiga toxin-producing *Escherichia coli*: an overview. Journal of Animal Science 85, E45–E62.

Hancock, D.D., Besser, T.E., Rice, D.H., Herriot, D.E., Tarr, P.I., 1997. A longitudinal study of *Escherichia coli* 0157 in fourteen cattle herds. Epidemiology and Infection 118, 193–195.

Hancock, D., Besser, T., LeJeune, J., Davis, M., Rice, D., 2001. The control of VTEC in the animal reservoir. International Journal of Food Microbiology 66, 71–78.

Hanna, S.S., Atkinson, R.M., Jones, T.F., Dunn, J.R., 2010. Shiga toxin-producing *Escherichia coli* (STEC) in Tennessee: surveillance and current clinical laboratory practices. Tennessee Medicine 103, 39–42.

Hara-Kudo, Y., Konuma, H., Nakagawa, H., Kumagai, S., 2000. *Escherichia coli* O26 detection from foods using an enrichment procedure and an immunomagnetic separation method. Letters in Applied Microbiology 30, 151–154.

Hazarika, R.A., Singh, D.K., Kapoor, K.N., Bhilegaonkar, K.N., Agarwal, R.K., Malik, S.V.S., 2003. Survivability of vero toxic *Escherichia coli* O111 against sodium benzoate and potassium sorbate added to simulating media for beef gravy. Journal of Veterinary Public Health 1, 103–112.

Herold, S., Karch, H., Schmidt, H., 2004. Shiga toxin-encoding bacteriophages—genomes in motion. International Journal of Medical Microbiology 294, 115–121.

Heuvelink, A.E., Van den Biggelaar, F.L., Zwartkruis-Nahuis, J., Herbes, R.G., Huyben, R., Nagelkerke, N., Melchers, W.J., Monnens, L.A., de Boer, E., 1998. Occurrence of verocytotoxin-producing *Escherichia coli* O157 on Dutch dairy farms. Journal of Clinical Microbiology 36, 3480–3487.

Hilborn, E.D., Mermin, J.H., Mshar, P.A., Hadler, J.L., Voetsch, A., Wojtkunski, C., Swartz, M., Mshar, R., Lambert-Fair, M.A., Farrar, J.A., Glynn, M.K., Slutsker, L., 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Archive of Internal Medicine 159, 1758–1764.

Hiramatsu, R., Matsumoto, M., Miwa, Y., Suzuki, Y., Saito, M., Miyazaki, Y., 2002. Characterization of Shiga toxin-producing *Escherichia coli* O26 strains and establishment of selective isolation media for these strains. Journal of Clinical Microbiology 40, 922–925.

Honish, L., Predy, G., Hislop, N., Chui, L., Kowalewska-Grochowska, K., Trottier, L., Kreplin, C., Zazulak, I., 2005. An outbreak of *E. coli* O157:H7 hemorrhagic colitis associated with unpasteurized Gouda cheese. Revue canadienne de Santé publique, 96, pp. 182–184.

Hudson, L.M., Chen, J., Hill, A.R., Griffiths, M.W., 1997. Bioluminescence: a rapid indicator of *Escherichia coli* O157:H7 in selected yogurt and cheese varieties. Journal of Food Protection 60, 891–897.

Hussein, H., Bollinger, L., 2005. Prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle. Journal of Food Protection 68, 2224–2241.

Hussein, H., Sakuma, T., 2005a. Prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products. Journal of Dairy Science 88, 450–465.

Hussein, H., Sakuma, T., 2005b. Shiga toxin-producing *Escherichia coli*: pre- and postharvest control measures to ensure safety of dairy cattle products. Journal of Food Protection 68 (1), 199–207 (2005).

ICSMF (International Commission on Microbiological Specifications for Foods), 1996. Micro-organisms in Foods 5: Characteristics of Microbial Pathogens. Blackie Academic & Professional, London; New York.

Imberechts, H., Pierard, D., Vanholme, L., Vereecken, K., 2007. Report on Zoonotic Agents in Belgium in 2007, pp. 41–44 (http://www.favv.be/publications-en/_documents/2006_Report-on-zoonotic-agents_en.pdf. Last accessed January 3rd 2012).

INVS (Institut de Veille Sanitaire), 2004. Investigation autour de deux cas de SHU à *E. coli* O157 dans les Vosges. (June 2004).

INVS (Institut de Veille Sanitaire), 2007. Epidémie d'infections à *E. coli* producteurs de Shiga-toxines non O157 liée à la consommation de camembert au lait cru, nord-ouest de la France, octobre-décembre 2005. Available at http://www.invs.sante.fr/publications/2008/epidemie_e_coli_camembert/rapport_epidemie_stec.pdf. Last accessed 21st August 2012.

ISO, 2001. Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Detection of *Escherichia coli* O157. (BS EN ISO 16654).

Ito, K., Iida, M., Yamazaki, M., Moriya, K., Moroishi, S., Yatsuyanagi, J., Kurazono, T., Hiruta, N., Ratchtrachenchai, O.A., 2007. Intimin types determined by heteroduplex mobility assay of intimin gene (*eae*)-positive *Escherichia coli* strains. Journal of Clinical Microbiology 45, 1038–1041.

Jacob, M.E., Callaway, T.R., Nagaraja, T.G., 2009. Dietary interactions and interventions affecting *Escherichia coli* O157 colonization and shedding in cattle. Foodborne Pathogens and Disease 6, 785–792.

Jasson, V., Rajkovic, A., Baert, L., Debevere, J., Uyttendaele, M., 2009. Comparison of enrichment conditions for rapid detection of low numbers of sublethally injured *Escherichia coli* O157 in food. Journal of Food Protection 72, 1862–1868.

Jayarao, B.M., Henning, D.R., 2001. Prevalence of foodborne pathogens in bulk tank milk. Journal of Dairy Science 84, 2157–2162.

Jayarao, B.M., Donaldson, S.C., Straley, B.A., Sawant, A.A., Hegde, N.V., Brown, J.L., 2006. A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. Journal of Dairy Science 89, 2451–2458.

Jenkins, C., Pearce, M.C., Smith, A.W., Knight, H.I., Shaw, D.J., Cheasty, T., Foster, G., Gunn, G.J., Dougan, G., Smith, H.R., Frankel, G., 2003. Detection of *Escherichia coli* serogroups O26, O103, O111 and O145 from bovine faeces using immunomagnetic separation and PCR/DNA probe techniques. Letters in Applied Microbiology 37, 207–212.

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Jensen, C., Ethelberg, S., Gervelmeyer, A., Nielsen, E.M., Olsen, K.E., Mølbak, K., 2006. First general outbreak of verocytotoxin-producing *Escherichia coli* O157 in Denmark. Eurosurveillance 11 (article 597, 2006-02-01).

Johnson, K.E., Thorpe, C.M., Sears, C.L., 2006. The emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli*. Clinical Infectious Diseases 43, 1587–1595.

Jordan, K.N., Maher, M.M., 2006. Sensitive detection of *Escherichia coli* O157:H7 by conventional plating techniques. Journal of Food Protection 69, 689–692.

Jordan, K.N., Oxford, L., O'Byrne, C.P., 1999. Survival of low-pH stress by *Escherichia coli* O157:H7: correlation between alterations in the cell envelope and increased acid tolerance. Applied and Environmental Microbiology 65, 3048–3055.

Judge, N.A., Mason, H.S., O'Brien, A.D., 2004. Plant cell-based intimin vaccine given orally to mice primed with intimin reduces time of *Escherichia coli* O157:H7 shedding in feces. Infection and Immunity 72, 168–175.

Kangethe, E.K., Onono, J.O., McDermott, B., Arimi, M., 2007. Isolation of *E. coli* O157:H7 from milk and cattle faeces from urban dairy farming and non dairy farming neighbour households in Dagoretti Division, Nairobi, Kenya: prevalence and risk factors. East African Medical Journal 84, S65–S75.

Kaper, J.B., Nataro, J.P., Mobley, H.L.T., 2004. Pathogenic *Escherichia coli*. Nature Reviews Microbiology 2, 123–140.

Karch, H., Bielaszewska, M., 2001. Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H(−) strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. Journal of Clinical Microbiology 39, 2043–2049.

Karch, H., Meyer, T., 1989. Single primer pair for amplifying segments of distinct Shiga-like-toxin genes by polymerase chain reaction. Journal of Clinical Microbiology 27, 2751–2757.

Karch, H., Tarr, P.I., Bielaszewska, M., 2005. Enterohaemorrhagic *Escherichia coli* in human medicine. International Journal of Medical Microbiology 295, 405–418.

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

Karmali, M.A., Gannon, V., Sargeant, J.M., 2010. Verocytotoxin-producing *Escherichia coli* (VTEC). Veterinary Microbiology 140, 360–370.

Karns, J.S., Van Kessel, J.S., McClusky, B.J., Perdue, M.L., 2007. Incidence of *Escherichia coli* O157:H7 and *E. coli* virulence factors in US bulk tank milk as determined by polymerase chain reaction. Journal of Dairy Science 90, 3212–3219.

Kaspar, C.W., Doyle, M.E., 2009. White Paper on Non-O157:H7 Shiga-toxin Producing *E. coli* from Meat and Non-meat Sources. American Meat Institute Foundation.

Kauppi, K.L., Tatini, S.R., Harrell, F., Feng, P., 1996. Influence of substrate and low temperature on growth and survival of verotoxigenic *Escherichia coli*. Food Microbiology 13, 397–405.

Kaur, J., Ledward, D.A., Park, R.W., Robson, R.L., 1998. Factors affecting the heat resistance of *Escherichia coli* O157:H7. Letters in Applied Microbiology 26, 325–330.

Keene, W.E., Hedberg, K., Herriott, D.E., Hancock, D.D., McKay, R.W., Barrett, T.J., Fleming, D.W., 1997. A prolonged outbreak of *Escherichia coli* O157:H7 infections caused by commercially distributed raw milk. Journal of Infectious Diseases 176, 815–818.

Khanna, R., Waechter, L., Sargeant, J., Clark, W.F., Garg, A.X., 2008. Environmental prevention of human disease from verocytotoxin-producing *Escherichia coli*. Nephrology, Dialysis, Transplantation 23, 1819–1822.

King, T., Lucchini, S., Hinton, J.C.D., Gobius, K., 2010. Transcriptomic analysis of *Escherichia coli* O157:H7 and K-12 cultures exposed to inorganic and organic acids in stationary phase reveals acidulant- and strain-specific acid tolerance responses. Applied and Environmental Microbiology 76, 6514–6528.

Konczy, P., Ziebell, K., Mascarenhas, M., Choi, A., Michaud, C., Kropinski, A.M., Whittam, T.S., Wickham, M., Finlay, B., Karmali, M.A., 2008. Genomic O island 122, locus for enterocyte effacement, and the evolution of virulent verocytotoxin-producing *Escherichia coli*. Journal of Bacteriology 190, 5832–5840.

Konowalchuk, J., Speirs, J.I., Stavric, S., 1977. Vero response to a cytotoxin of *Escherichia coli*. Infection and Immunity 18, 775–779.

Kousta, M., Mataragas, M., Skandamis, P., Drosinos, E.H., 2010. Prevalence and sources of cheese contamination with pathogens at farm and processing levels. Food Control 21, 805–815.

Kudva, I., Hatfield, P.G., Hovde, C.J., 1996. *Escherichia coli* O157:H7 in microbial flora of sheep. Journal of Clinical Microbiology 34, 431–433.

Kudva, I., Hatfield, P.G., Hovde, C.J., 1997. Characterization of *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* serotypes isolated from sheep. Journal of Clinical Microbiology 35, 892–899.

La Ragione, R.M., Best, A., Woodward, M.J., Wales, A.D., 2009. *Escherichia coli* O157:H7 colonization in small domestic ruminants. FEMS Microbiology Reviews 33, 394–410.

Lahti, E., Eklund, M., Ruuru, P., Siitonen, A., Rantala, L., Nuorti, P., Honkanen-Busalski, T., 2002. Use of phenotyping and genotyping to verify transmission of *Escherichia coli* O157:H7 from dairy farms. European Journal of Clinical Microbiology and Infectious Diseases 21, 189–195.

Lahti, E., Ruoho, O., Rantala, L., Hänninen, M.L., Honkanen-Buzalski, T., 2003. Longitudinal study of *Escherichia coli* O157 in a cattle finishing unit. Applied and Environmental Microbiology 69, 554–561.

Laing, C.R., Buchanan, C., Taboada, E.N., Zhang, Y., Karmali, M.A., Thomas, J.E., Gannon, V.P., 2009. In silico genomic analyses reveal three distinct lineages of *Escherichia coli* O157: H7, one of which is associated with hyper-virulence. BMC Genomics 10, 287.

Leistner, L., 1995. Principles and applications of hurdle technology. In: Gould, G.W. (Ed.), New Methods of Food Preservation. Blackie Academic and Professional, London, pp. 1–21.

LeJeune, J.T., Wetzel, A.N., 2007. Preharvest control of *Escherichia coli* O157 in cattle. Journal of Animal Science 85, 73–80.

LeJeune, J.T., Besser, T.E., Hancock, D.D., 2001. Cattle water troughs as reservoirs of *Escherichia coli* O157. Applied and Environmental Microbiology 67, 3053–3057.

LeJeune, J.T., Besser, T.E., Rice, D.H., Berg, J.L., Stilborn, R.P., Hancock, D.D., 2004. Longitudinal study of fecal shedding of *Escherichia coli* 0157:H7 in feedlot cattle: predominance and persistence of specific clonal types despite massive cattle population turnover. Applied and Environmental Microbiology 70, 377–384.

LeJeune, J.T., Hancock, D., Wasteson, Y., Skjerve, E., Urdahl, A.M., 2006. Comparison of *E. coli* O157 and Shiga toxin-encoding genes (stx) prevalence between Ohio, USA and Norwegian dairy cattle. International Journal of Food Microbiology 109, 19–24.

Liebana, E., Smith, R.P., Batchelor, M., McLaren, I., Cassar, C., Clifton-Hadley, F.A., Paiba, G.A., 2005. Persistence of *Escherichia coli* O157 isolates on bovine farms in England and Wales. Journal of Clinical Microbiology 43, 898–902.

Lin, A., Sultan, O., Lau, H.K., Wong, E., Hartman, G., Lauzon, C.R., 2011. O serogroup specific real time PCR assays for the detection and identification of nine clinically relevant non-O157 STECs. Food Microbiology 28, 478–483.

Lindblad, M., Lindqvist, R., 2010. Modelling time to growth of *Escherichia coli* as a function of water activity and undissociated lactic acid. Letters in Applied Microbiology 50, 308–313.

Line, J.E., Fain Jr., A.R., Moran, A.B., Martin, L.M., Lechowich, R.V., Carosella, J.M., Brown, W.L., 1991. Lethality of heat to *Escherichia coli* O157:H7: D-value and Z-value determinations in ground beef. Journal of Food Protection 54, 762–766.

Liptakova, A., Siegfried, L., Rosocha, J., Podracka, L., Bogyiova, E., Kotulova, D., 2004. A family outbreak of haemolytic uraemic syndrome and haemorrhagic colitis caused by verocytotoxigenic *Escherichia coli* O157 from unpasteurised cow's milk in Slovakia. Clinical Microbiology and Infection 10, 576–578.

Lira, W.M., Macedo, C., Marin, J.M., 2004. The incidence of Shiga toxin-producing *Escherichia coli* in cattle with mastitis in Brazil. Journal of Applied Microbiology 97, 831–866.

Loneragan, G.H., Brashears, M.M., 2005. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. Meat Science 71, 72–78.

Lorusso, V., Dambrosio, A., Quaglia, N.C., Parisi, A., La Salandra, G., Lucifora, G., Mula, G., Virgilio, S., Carosielli, L., Rella, A., Dario, M., Normanno, G., 2009. Verocytotoxin-producing *Escherichia coli* O26 in raw water buffalo (*Bubalus bubalis*) milk products in Italy. Journal of Food Protection 72, 1705–1708.

Lynch, M.J., O'Connor, L., Fox, E.M., Jordan, K., Murphy, M., 2012. Verocytotoxigenic *Escherichia coli* O157, O111, O26, O103, O145 in Irish dairy cattle and raw milk: prevalence and epidemiology of emergent stains. Zoonoses and Public Health 59, 264–271. http://dx.doi.org/10.1111/j.1863-2378.2011.01443.x.

Madic, J., Lecureuil, C., Dilasser, F., Derzelle, S., Jamet, E., Fach, P., Auvray, F., 2009. Screening of food raw materials for the presence of Shiga toxin-producing *Escherichia coli* O91:H21. Letters in Applied Microbiology 48, 447–451.

Madic, J., Peytavin de Garam, C., Vingadassalon, N., Oswald, E., Fach, P., Jamet, E., Auvray, F., 2010. Simplex and multiplex real-time PCR assays for the detection of flagellar (H-antigen) fliC alleles and intimin (eae) variants associated with enterohaemorrhagic *Escherichia coli* (EHEC) serotypes O26:H11, O103:H2, O111: H8, O145:H28 and O157:H7. Journal of Applied Microbiology 109, 1696–1705.

Madic, J., Vingadassalon, N., de Garam, C.P., Marault, M., Scheutz, F., Brugere, H., Jamet, E., Auvray, F., 2011. Detection of Shiga toxin-producing *Escherichia coli* serotypes O26: H11, O103:H2, O111:H8, O145:H28, and O157:H7 in raw-milk cheeses by using multiplex real-time PCR. Applied and Environmental Microbiology 77, 2035–2041.

Mahaut, M., Jeantet, R., Brulé, G., 2000. Initiation à la technologie fromagère. TEC&DOC ed, Paris.

Maher, M.M., Jordan, K.N., Upton, M.E., Coffey, A., 2001. Growth and survival of *E. coli* O157:H7 during the manufacture and ripening of a smear-ripened cheese produced from raw milk. Journal of Applied Microbiology 90, 201–207.

Manna, S.K., Manna, C., Batabyal, K., Das, B., Golder, D., Chattopadhyay, S., Biswas, B.K., 2010. Serogroup distribution and virulence characteristics of sorbitol-negative *Escherichia coli* from food and cattle stool. Journal of Applied Microbiology 108, 658–665.

Manning, S.D., Motiwala, A.S., Springman, A.C., Qi, W., Lacher, D.W., Ouellette, L.M., Mladonicky, J.M., Somsel, P., Rudrik, J.T., Dietrich, S.E., Zhang, W., Swaminathan, B., Alland, D., Whittam, T.S., 2008. Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. Proceedings of the National Academy of Sciences of the United States of America 105, 4868–4873.

MAPAQ (Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec), 2004. Mise en garde - Onze personnes malades à la suite de la consommation de fromages au lait cru fabriqués à la ferme. Press Release, 2004-07-14.

March, S.B., Ratnam, S., 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. Journal of Clinical Microbiology 23, 869–872.

Marler, B., 2009. 2008 Raw Goat Milk *E. coli* O157:H7 Outbreak Linked to Dairy and Retail. Marlor Blog. (2009-12-16, http://www.marlerblog.com/legal-cases/2008-raw-goat-milk-e-coli-o157h7-outbreak-linked-to-dairy-and-retail/. Last accessed 23 April 2012).

Marler, B., 2010. Raw Milk *E. coli* O157:H7 Outbreak Strikes Minnesota. Seventh Outbreak in 2010. Marler Blog. (2010-05-28, http://www.marlerblog.com/legal-cases/raw-milk-e-coli-o157h7-outbreak-strikes-minnesota-seventh-outbreak-in-2010/. Last accessed 23 April 2012).

Marouani-Gadri, N., Augier, G., Carpentier, B., 2009. Characterization of bacterial strains isolated from a beef-processing plant following cleaning and disinfection — influence of isolated strains on biofilm formation by Sakaï and EDL 933 *E. coli* O157:H7. International Journal of Food Microbiology 133, 62–67.

Márquez, J.G., García, C.E.R., 2007. Pathogenic microflora found in white "telita" cheese made in four states of Venezuela. Anales Venezolanos de Nutricion 20, 17–21.

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Martin, M.L., Shipman, L.D., Wells, J.G., Potter, M.E., Hedberg, K., Wachsmuth, I.K., Tauxe, R.V., Davis, J.P., Arnoldi, J., Tilleli, J., 1986. Isolation of *Escherichia coli* O157: H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome. The Lancet 328, 1043.

Martucciello, A., Astarita, S., Ruggia, A., Alfano, D., Morena, C., Galiero, G., 2008. A survey on *Salmonella* spp., *Campylobacter* spp., and *Escherichia coli* O157 presence in Campania buffalo mozzarella cheese by real-time PCR. Industrie Alimentari 47, 1–2.

Mathusa, E.C., Chen, Y., Enache, E., Hontz, L., 2010. Non-O157 Shiga toxin-producing *Escherichia coli* in foods. Journal of Food Protection 73, 1721–1736.

Matthews, K.R., Murdough, P.A., Bramley, A.J., 1997. Invasion of bovine epithelial cells by verocytotoxin-producing *Escherichia coli* O157:H7. Journal of Applied Microbiology 82, 197–203.

Matthews, L., Low, J.C., Gally, D.L., Pearce, M.C., Mellor, D.J., Heesterbeek, J.A., Chase-Topping, M., Naylor, S.W., Shaw, D.J., Reid, S.W., Gunn, G.J., Woolhouse, M.E., 2006. Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. Proceedings of the National Academy of Sciences of the United States of America 103, 547–552.

McGee, P., Scott, L., Sheridan, J.J., Earley, B., Leonard, N., 2004. Horizontal transmission of *Escherichia coli* O157:H7 during cattle housing. Journal of Food Protection 12, 2651–2656.

McIntyre, L., Fung, J., Paccagnella, A., Isacc-Renton, J., Rockwell, F., Emerson, B., Preston, T., 2002. *Escherichia coli* O157 outbreak associated with the ingestion of unpasteurized goat's milk in British Columbia, 2001. Canadian Communicable Disease Report 28, 6–8.

McKee, R., Madden, R.H., Gilmour, A., 2003. The occurrence of verocytotoxin-producing *Escherichia coli* in dairy and meat processing environments. Journal of Food Protection 66, 1576–1580.

McNeilly, T.N., Naylor, S.W., Mahajan, A., Mitchell, M.C., McAteer, S., Deane, D., Smith, D.G.E., Low, J.C., Gally, D.L., Huntley, J.F., 2008. *Escherichia coli* O157:H7 colonization in cattle following systemic and mucosal immunization with purified H7 flagellin. Infection and Immunity 76, 2594–2602.

McNeilly, T.N., Mitchell, M.C., Rosser, T., McAteer, S., Low, J.C., Smith, D.G., Huntley, J.F., Mahajan, A., Gally, D.L., 2010. Immunization of cattle with a combination of purified intimin-531, EspA and Tir significantly reduces shedding of *Escherichia coli* O157:H7 following oral challenge. Vaccine 28, 1422–1428.

McWilliam Leitch, E.C., Stewart, C.S., 2002a. Susceptibility of *Escherichia coli* O157 and non-O157 isolates to lactate. Letters in Applied Microbiology 35, 176–180.

McWilliam Leitch, E.C., Stewart, C.S., 2002b. *Escherichia coli* O157 and non-O157 isolates are more susceptible to L-lactate than to D-lactate. Applied and Environmental Microbiology 68, 4676–4678.

Meals, D.W., Braun, D.C., 2006. Demonstration of methods to reduce *E. coli* runoff from dairy manure application sites. Journal of Environmental Quality 35, 1088–1100.

Mechie, S.C., Chapman, P.A., Siddons, C.A., 1997. A fifteen month study of *Escherichia coli* O157:H7 in a dairy herd. Epidemiology and Infection 118, 17–25.

Mellmann, A., Harmsen, D., Cummings, C.A., Zentz, E.B., Leopold, S.R., Rico, A., Prior, K., Szczepanowski, R., Ji, Y.M., Zhang, W.L., McLaughlin, S.F., Stephen, F., Henkhaus, J.K., Leopold, B., Bielaszewska, M., Prager, R., Brzoska, P.M., Moore, R.L., Guenther, S., Rothberg, J.M., Karch, H., 2011. Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology. PLoS One 6 (Article Number: e22751).

Meng, J., Doyle, M.P., Zhao, T., Zhao, S., 2007. Enterohemorrhagic *Escherichia coli*, In: Doyle, M.P., Beuchat, L.R. (Eds.), Food Microbiology — Fundamentals and Frontiers, 3rd edition. American Society for Microbiology, Washington D.C., pp. 249–269.

Messelhäusser, U., Beck, H., Gallien, P., Schalch, B., Busch, U., 2008. Presence of Shiga toxin-producing *Escherichia coli* and thermophilic *Campylobacter* spp. in cattle, food and water sources on Alpine pastures in Bavaria. Archiv für Lebensmittelhygiene 59, 103–106.

Meyer-Broseta, S., Bastian, S.N., Arné, P.D., Cerf, O., Sanaa, M., 2001. Review of epidemiological surveys on the prevalence of contamination of healthy cattle with *Escherichia coli* serogroup O157:H7. International Journal of Hygiene and Environmental Health 203, 347–361.

Miko, A., Lindstedt, B.A., Brandal, L.T., Lobersli, I., Beutin, L., 2010. Evaluation of multiple-locus variable number of tandem-repeats analysis (MLVA) as a method for identification of clonal groups among enteropathogenic, enterohaemorrhagic and avirulent *Escherichia coli* O26 strains. FEMS Microbiology Letters 303, 137–146.

Millet, L., Saubusse, M., Didienne, R., Tessier, L., Montel, M.C., 2006. Control of *Listeria monocytogenes* in raw-milk cheeses. International Journal of Food Microbiology 108, 105–114.

Minnesota Department of Health, 2010. Three more *E. coli* cases linked to raw milk from farm. News release, 2010-06-11, http://www.health.state.mn.us/news/pressrel/2010/ecoli061110.html. Last accessed 23rd April 2012.

Monday, S.R., Beisaw, A., Feng, P.C., 2007. Identification of Shiga toxigenic *Escherichia coli* seropathotypes A and B by multiplex PCR. Molecular and Cellular Probes 21, 308–311.

Montet, M., Jamet, E., Ganet, S., Dizin, M., Miszczycha, S., Dunière, L., Thevenot, D., Vernozy-Rozand, C., 2009. Growth and survival of acid-resistant and non-acid-resistant Shiga-toxin-producing *Escherichia coli* strains during the manufacture and ripening of Camembert cheese. International Journal of Microbiology 653481, 1–10 (article ID).

Mora, A., Blanco, M., Blanco, J.E., 2004. Phages types and genotypes of Shiga toxin-producing *Escherichia coli* O157:H7 isolates from humans and animals in Spain: identification and characterization of two predominating phage types (PT2 and PT8). Journal of Clinical Microbiology 42, 4007–4015.

Mora, A., Leon, S.L., Blanco, M., Blanco, J.E., Lopez, C., Dahbi, G., Echeita, A., Gonzalez, E.A., Blanco, J., 2007. Phage types, virulence genes and PFGE profiles of Shiga toxin-producing *Escherichia coli* O157:H7 isolated from raw beef, soft cheese and vegetables in Lima (Peru). International Journal of Food Microbiology 114, 204–210.

Morgan, D., Newman, C.P., Hutchinson, D.N., Waiker, A.M., Rowe, B., Majid, F., 1993. Verotoxin producing *Escherichia coli* O157 infections associated with the consumption of yoghurt. Epidemiology and Infection 111, 181–187.

Morgan, F., Bonnin, V., Mallereau, M.-P., Perrin, G., 2001. Survival of *Listeria monocytogenes* during manufacture, ripening and storage of soft lactic cheese made from raw goat milk. International Journal of Food Microbiology 64, 217–221.

Muehlherr, J.E., Zweifel, C., Corti, S., Blanco, J.E., Stephan, R., 2003. Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. Journal of Dairy Science 86, 3849–3856.

Murano, E.A., Pierson, M.D., 1992. Effect of heat shock and growth atmosphere on the heat resistance of *Escherichia coli* O157:H7. Journal of Food Protection 55, 171–175.

Murinda, S.E., Nguyen, L.T., Ivey, S.J., Gillespie, B.E., Almeida, R.A., Draughon, F.A., Oliver, S.P., 2002. Prevalence and molecular characterization of *Escherichia coli* O157:H7 in bulk tank milk and fecal samples from cull cows: a 12-month survey of dairy farms in east Tennessee. Journal of Food Protection 65, 752–759.

Murphy, M., Buckley, J.F., Whyte, P., Mahony, M.O., Anderson, W., Wall, P.G., Fanning, S., 2007. Surveillance of dairy production holdings supplying raw milk to the farmhouse cheese sector for *Escherichia coli* O157, O26 and O111. Zoonoses and Public Health 54, 358–365.

Nadala, C., Samadpour, M., 2011. Development of a non-O157:H7 enterohemorrhagic *Escherichia coli* lateral flow device. International Association of Food Protection Annual Meeting, Milwaukee, USA, pp. P3–P11.

Nastasijevic, I., Mitrovic, R., Buncic, S., 2008. Occurrence of *Escherichia coli* O157 on hides of slaughtered cattle. Letters in Applied Microbiology 46, 126–131.

Nataro, J.P., Kaper, J.B., 1998. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews 11, 142–201.

Naylor, S.W., Flockhart, A., Nart, P., Smith, D.G., Huntley, J., Gally, D.L., Low, J.C., 2007. Shedding of *Escherichia coli* O157:H7 in calves is reduced by prior colonization with the homologous strain. Applied and Environmental Microbiology 73, 3765–3767.

Nielsen, E.M., Andersen, M.T., 2003. Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5′ nuclease PCR assay. Journal of Clinical Microbiology 41, 2884–2893.

Nielsen, E.M., Skov, M.N., Madsen, J.J., Lodal, J., Jespersen, J.B., Baggesen, D.L., 2004. Verocytotoxin-producing *Escherichia coli* in wild birds and rodents in close proximity to farms. Applied and Environmental Microbiology 70, 6944–6947.

Noller, A.C., McEllistrem, M.C., Pacheco, A.G., Boxrud, D.J., Harrison, L.H., 2003. Multilocus variable-number tandem repeat analysis distinguishes outbreak and sporadic *Escherichia coli* O157:H7 isolates. Journal of Clinical Microbiology 41, 5389–5397.

Nooitgedagt, A.J., Hartog, B.J., 1988. A survey of the microbiological quality of Brie and Camembert cheese. Netherlands Milk and Dairy Journal 42, 57–72.

O'Hanlon, K.A., Catarame, T.M., Duffy, G., Blair, I.S., McDowell, D.A., 2004. RAPID detection and quantification of *E. coli* O157/O26/O111 in minced beef by real-time PCR. Journal of Applied Microbiology 96, 1013–1023.

Oksuz, O., Arici, M., Kurultay, S., Gumus, T., 2004. Incidence of *Escherichia coli* O157 in raw milk and white pickled cheese manufactured from raw milk in Turkey. Food Control 15, 453–456.

Orden, J.A., Ruiz-Santa-Quiteria, J.A., Blanco, M., Blanco, J.E., Mora, A., Cid, D., Gonzalez, E.A., Blanco, J., De La Fuente, R., 2003. Prevalence and characterization of vero cytotoxin-producing *Escherichia coli* isolated from diarrhoeic and healthy sheep and goats. Epidemiology and Infection 130, 313–321.

Orden, J.A., Cortes, C., Horcajo, P., De la Fuente, R., Blanco, J.E., Mora, A., Lopez, C., Blanco, J., Contreras, A., Sanchez, A., Corrales, J.C., Domınguez-Bernal, G., 2008. A longitudinal study of verotoxin-producing *Escherichia coli* in two dairy goat herds. Veterinary Microbiology 132, 428–434.

Oswald, E., Schmidt, H., Morabito, S., Karch, H., Marches, O., Caprioli, A., 2000. Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: characterization of a new intimin variant. Infection and Immunity 68, 64–71.

Papageorgiou, D.K., Marth, E.H., 1989. Fate of *Listeria monocytogenes* during the manufacture and ripening of blue cheese. Journal of Food Protection 52, 459–465.

Parisi, A., Miccolupo, A., Santagada, G., Pedarra, C., Dambrosio, A., Normanno, G., 2010. Detection of verocytotoxin-producing *Escherichia coli* (VTEC) in minced beef and raw milk by colony hybridization. Food Control 21, 770–773.

Paton, A.W., Paton, J.C., 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. Journal of Clinical Microbiology 36, 598–602.

Paton, A.W., Woodrow, M.C., Doyle, R.M., Lanser, J.A., Paton, J.C., 1999. Molecular characterization of a Shiga toxigenic *Escherichia coli* O113:H21 strain lacking eae responsible for a cluster of cases of hemolytic–uremic syndrome. Journal of Clinical Microbiology 37, 3357–3361.

Peng, S., Tasara, T., Hummerjohann, J., Stephan, R., 2011. An overview of molecular stress response mechanisms in *Escherichia coli* contributing to survival of Shiga toxin-producing *Escherichia coli* during raw milk cheese production. Journal of Food Protection 74, 849–864.

Perelle, S., Dilasser, F., Grout, J., Fach, P., 2004. Detection by 5′-nuclease PCR of Shiga-toxin producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157: H7, associated with the world's most frequent clinical cases. Molecular and Cellular Probes 18, 185–192.

Perelle, S., Dilasser, F., Grout, J., Fach, P., 2007. Screening food raw materials for the presence of the world's most frequent clinical cases of Shiga toxin-encoding *Escherichia coli* O26, O103, O111, O145 and O157. International Journal of Food Microbiology 113, 284–288.

Persson, S., Olsen, K.E., Ethelberg, S., Scheutz, F., 2007. Subtyping method for *Escherichia coli* Shiga toxin (verocytotoxin) 2 variants and correlations to clinical manifestations. Journal of Clinical Microbiology 45, 2020–2024.

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Peterson, R.E., Klopfenstein, T.J., Moxley, R.A., Erickson, G.E., Hinkley, S., Bretschneider, G., Berberov, E.M., Rogan, D., Smith, D.R., 2007a. Effect of a vaccine product containing type III secreted proteins on the probability of *Escherichia coli* O157:H7 fecal shedding and mucosal colonization in feedlot cattle. Journal of Food Protection 70, 2568–2577.

Peterson, R.E., Klopfenstein, T.J., Moxley, R.A., Erickson, G.E., Hinkley, S., Rogan, D., Smith, D.R., 2007b. Efficacy of dose regimen and observation of herd immunity from a vaccine against *Escherichia coli* O157:H7 for feedlot cattle. Journal of Food Protection 70, 2561–2567.

Picozzi, C., Foschino, R., Heuvelink, A., Beumer, R., 2005. Phenotypic and genotypic characterization of sorbitol negative or slow-fermenting (suspected O157) *Escherichia coli* isolated from milk samples in Lombardy region. Letters in Applied Microbiology 40, 491–496.

Pontello, M., Bersani, C., Colmegna, S., Cantoni, C., Mammina, C., Rizzi, M., Barri, A., Nozza, L., 2003. Verocytotoxin-producing *Escherichia coli* in foodstuffs of animal origin. European Journal of Epidemiology 18, 157–160.

Possé, B., De Zutter, L., Heyndrickx, M., Herman, L., 2008a. Novel differential and confirmation plating media for Shiga toxin-producing *Escherichia coli* serotypes O26, O103, O111, O145 and sorbitol-positive and -negative O157. FEMS Microbiology Letters 282, 124–131.

Possé, B., De Zutter, L., Heyndrickx, M., Herman, L., 2008b. Quantitative isolation efficiency of O26, O103, O111, O145 and O157 STEC serotypes from artificially contaminated food and cattle faeces samples using a new isolation protocol. Journal of Applied Microbiology 105, 227–235.

Pradel, N., Livrelli, V., De Champs, C., Palcoux, J.B., Reynaud, A., Scheutz, F., Sirot, J., Joly, B., Forestier, C., 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. Journal of Clinical Microbiology 38, 1023–1031.

Pradel, N., Bertin, Y., Martin, C., Livrelli, V., 2008. Molecular analysis of Shiga toxin-producing *Escherichia coli* strains isolated from hemolytic–uremic syndrome patients and dairy samples in France. Applied and Environmental Microbiology 74, 2118–2128.

Rahn, K., Renwick, S.A., Johnson, R.P., Wilson, J.B., Clarke, R.C., Alves, D., McEwen, S., Lior, H., Spika, J., 1997. Persistence of *Escherichia coli* O157:H7 in dairy cattle and the dairy farm environment. Epidemiology and Infection 119, 251–259.

Ramsaran, H., Chen, J., Brunke, B., Hill, A., Griffiths, M.W., 1998. Survival of bioluminescent *Listeria monocytogenes* and *Escherichia coli* O157:H7 in soft cheeses. Journal of Dairy Science 81, 1810–1817.

Raynal-Ljutovac, K., Barral, J., Poutrel, B., Guillet, I., Boivin, J., Gaborit, P., Dirberg, R., De Crémoux, R., 2006. Recherche d'écosystèmes microbiens inhibiteurs de la croissance de *S. aureus* dans le lait de chèvre. 13èmes Rencontres Recherche Ruminants, pp. 415–418.

Raynal-Ljutovac, K., Barral, J., Gaborit, P., Guillet, I., Boivin, J., Dirberg, R., De Crémoux, R., Beuvier, E., Poutrel, B., 2008. Inhibition naturelle de la croissance de *S. aureus* dans les fromages au lait cru de chèvre. 15èmes Rencontres Recherche Ruminants, p. 115.

Raynaud, S., Vernozy-Rozand, C., Boscher, P., Picant, P., Mathieu, B., Degand, C., Poutrel, B., Heuchel, V., Chatelin, Y.M., 2005. Prévalence, origine, circulation et persistance des *Escherichia coli* producteurs de Shiga-toxines (STEC) dans les élevages bovins Français. 12èmes Rencontres Recherche Ruminants, pp. 379–382.

Raynaud, S., Boscher, P., Picant, P., Mathieu, B., Degand, C., Poutreil, B., Heuchel, V., Chaltelain, Y.M., Vernozy-Rozand, C., 2006. Prévalence, origine, circulation et persistence des *Escherichia coli* producteurs de shiga-toxines (STEC) dans les élevages bovins français. Bulletin Epidémiologique 21, 1–3.

Reischl, U., Youssef, M.T., Kilwinski, J., Lehn, N., Zhang, W.L., Karch, H., Strockbine, N.A., 2002. Real-time fluorescence PCR assays for detection and characterization of Shiga toxin, intimin, and enterohemolysin genes from Shiga toxin-producing *Escherichia coli*. Journal of Clinical Microbiology 40, 2555–2565.

Reitsma, C.J., Henning, D.R., 1996. Survival of enterohemorrhagic *Escherichia coli* O157:H7 during the manufacture and curing of Cheddar cheese. Journal of Food Protection 59, 460–464.

Rey, J., Sanche, S., Blanco, J.E., De Mendoza, J.H., De Mendoza, M.H., Garcia, A., Gil, C., Tejero, N., Rubio, R., Alonso, J.M., 2006. Prevalence, serotypes and virulence genes of Shiga toxin-producing *Escherichia coli* isolated from ovine and caprine milk and other dairy products in Spain. International Journal of Food Microbiology 107, 212–217.

RKI (Robert Koch Institut), 2008. Zum Auftreten mehrerer EHEC-Infektionen nach Rohmilchverzehr in einem Ferienlager. Epidemiologisches Bulletin 11, 16–18.

Rodriguez, E., Arques, J.A., Nunez, M., Gaya, P., Medina, M., 2005. Combined effect of high pressure treatments and bacteriocin producing lactic acid bacteria on inactivation of in raw milk cheese. Applied and Environmental Microbiology 71, 3399–3404.

Rohde, H., Qin, J., Cui, Y., Li, D., Loman, N.J., Hentschke, M., Chen, W., Pu, F., Peng, Y., Li, J., et al., 2011. Open-source genomic analysis of Shiga-toxin-producing *E. coli* O104:H4. The New England Journal of Medicine 365, 718–724.

Roldán, M.L., Chinen, I., Otero, J.L., Miliwebsky, E.S., Alfaro, N., Burns, P., Rivas, M., 2007. Isolation, characterization and typing of *Escherichia coli* 0157:H7 strains from beef products and milk. Revista Argentina de Microbiologia 39, 113–119.

Roopnarine, R.R., Ammons, D., Rampersad, J., Adesiyun, A.A., 2007. Occurrence and characterization of verocytotoxigenic *Escherichia coli* (VTEC) strains from dairy farms in Trinidad. Zoonoses and Public Health 54, 78–85.

Rostagno, M.H., 2009. Can stress in farm animals increase food safety risk? Foodborne Pathogens and Disease 6, 767–776.

Rothschild, M., 2010. Sally Jackson cheese confirmed as outbreak source. Food Safety News-12-21, http://beforeitsnews.com/story/319/970/Sally_Jackson_Cheese_Confirmed_as_Outbreak_Source.html. Last accessed 17th October 2011.

Rowe, M.T., Kirk, R., 1999. An investigation into the phenomenon of cross-protection in *Escherichia coli* O157:H7. Food Microbiology 16, 157–164.

Rozema, E.A., Stephens, T.P., Bach, S.J., Okine, E.K., Johnson, R.P., Stanford, K., McAllister, T.A., 2009. Oral and rectal administration of bacteriophages for control of *Escherichia coli* O157:H7 in feedlot cattle. Journal of Food Protection 72, 241–250.

Rump, L.V., Strain, E.A., Cao, G., Allard, M.W., Fischer, M., Brown, E.W., Gonzalez-Escalona, N., 2011. Draft genome sequences of six *Escherichia coli* isolates from the stepwise model of emergence of *Escherichia coli* O157:H7. Journal of Bacteriology 193, 2058–2059.

Ryser, E.T., Marth, E.H., 1987a. Fate of *Listeria monocytogenes* during the manufacture and ripening of camembert cheese. Journal of Food Protection 50, 372–378.

Ryser, E.T., Marth, E.H., 1987b. Behaviour of *Listeria monocytogenes* during the manufacture and ripening of camembert cheese. Journal of Food Protection 50, 7–13.

Ryu, J., Beuchat, L., 1998. Influence of acid tolerance responses on survival, growth, and thermal cross-protection of *Escherichia coli* O157:H7 in acidified media and fruit juices. International Journal of Food Microbiology 45, 185–193.

Ryu, J.H., Beuchat, L.R., 1999. Changes in heat tolerance of *Escherichia coli* O157:H7 after exposure to acidic environments. Food Microbiology 16, 317–324.

Ryu, J.H., Beuchat, L.R., 2005. Biofilm formation by *Escherichia coli* O157:H7 on stainless steel: effect of exopolysaccharide and curli production on Its resistance to chlorine. Applied and Environmental Microbiology 71, 247–254.

Ryu, J.H., Kim, H., Frank, J.F., Beuchat, L.R., 2004. Attachment and biofilm formation on stainless steel by *Escherichia coli* O157:H7 as affected by curli production. Letters in Applied Microbiology 39, 359–362.

Salter, M.A., Ratkowsky, D.A., Ross, T., McMeekin, T.A., 2000. Modelling the combined temperature and salt (NaCl) limits for growth of a pathogenic *Escherichia coli* strain using nonlinear logistic regression. International Journal of Food Microbiology 61, 159–167.

Sargeant, J.M., Amezcua, M.R., Rajic, A., Waddell, L., 2007. Pre-harvest interventions to reduce the shedding of *E. coli* O157 in the faeces of domestic ruminants: a systematic review. Zoonoses and Public Health 54, 260–277.

Sartz, L., De Jong, B., Hjertqvist, M., Plym-Forshell, L., Alsterlund, R., Löfdahl, S., Osterman, B., Ståhl, A., Eriksson, E., Hansson, H., Karpman, D., 2008. An outbreak of *Escherichia coli* O157:H7 infection in southern Sweden associated with consumption of fermented sausage; aspects of sausage production that increase the risk of contamination. Epidemiology and Infection 136, 370–380.

Scheutz, F., Strockbine, N.A., 2005. Escherichia. In: Garrity, G.M., Brenner, D.J., Krieg, N.R., Staley, J.T. (Eds.), Bergey's Manual of Systematic Bacteriology. Springer, New York, NY, pp. 607–624.

Scheutz, F., Moller Nielsen, E., Frimodt-Moller, F., Boisen, N., Morabito, S., Tozzoli, R., Nataro, J.P., Caprioli, A., 2011. Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. Eurosurveillance 16 (article 19889).

Schlesser, J.E., Gerdes, R., Ravishankar, S., Madsen, K., Mowbray, J., Teo, A.Y.L., 2006. Survival of a five-strain cocktail of *Escherichia coli* O157:H7 during the 60-day aging period of Cheddar cheese made from unpasteurized milk. Journal of Food Protection 69, 990–998.

Schmidt, H., 2001. Shiga-toxin-converting bacteriophages. Research in Microbiology 152, 687–695.

Schouten, J.M., Graat, E.A.M., Frankena, K., van de Giessen, A.W., van der Zwaluw, W.K., de Jong, M.C.M., 2005. A longitudinal study of *Escherichia coli* O157 in cattle of a Dutch dairy farm and in the farm environment. Veterinary Microbiology 107, 193–204.

Sehgal, R., Kumar, Y., Kumar, S., 2008. Prevalence and geographical distribution of *Escherichia coli* O157 in India: a 10-year survey. Transactions of the Royal Society of Tropical Medicine and Hygiene 102, 380–383.

Semanchek, J.J., Golden, D.A., 1996. Survival of *Escherichia coli* O157:H7 during fermentation of apple cider. Journal of Food Protection 59, 1256–1259.

Semanchek, J.J., Golden, D.A., 1998. Influence of growth temperature on inactivation and injury of *Escherichia coli* O157:H7 by heat, acid, and freezing. Journal of Food Protection 61, 395–401.

Sharma, M., Ryu, J.H., Beuchat, L.R., 2005. Inactivation of *Escherichia coli* O157:H7 in biofilm on stainless steel by treatment with an alkaline cleaner and a bacteriophage. Journal of Applied Microbiology 99, 449–459.

Shere, J.A., Bartlett, K.J., Kaspar, C.W., 1998. Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin. Applied and Environmental Microbiology 64, 1390–1399.

Shere, J.A., Kaspar, C.W., Bartlett, K.J., Linden, S.E., Norell, B., Francey, S., Schaefer, D.M., 2002. Shedding of *Escherichia coli* 0157:H7 in dairy cattle housed in a confined environment following waterborne inoculation. Applied and Environmental Microbiology 68, 1947–1954.

Singh, R.S., Ranganathan, B., 1980. Heat resistance of *Escherichia coli* in cow and buffalo milk. Journal of Food Protection 43, 376–380.

Smith, D., Blackford, M., Younts, S., Moxley, R., Gray, J., Hungerford, L., Milton, T., Klopfenstein, T., 2001. Ecological relationships between the prevalence of cattle shedding *Escherichia coli* O157:H7 and characteristics of the cattle or conditions of the feedlot pen. Journal of Food Protection 64, 1899–1903.

Smith, D.R., Moxley, R.A., Peterson, R.E., Klopfenstein, T.J., Erickson, G.E., Clowser, S.L., 2008. A two-dose regimen of a vaccine against *Escherichia coli* O157:H7 type III secreted proteins reduced environmental transmission of the agent in a large-scale commercial Beef feedlot clinical trial. Foodborne Pathogens and Disease 5, 589–598.

Solecki, O., MacRae, M., Strachan, N., Lindstedt, B.A., Odgen, I., 2009. *E. coli* O157 from sheep in Northeast Scotland: prevalence, concentration shed and molecular characterization by multilocus variable tandem repeat analysis. Foodborne Pathogens and Disease 6, 849–854.

Solomakos, N., Govaris, A., Angelidis, A.S., Pournaras, S., Rodi Burriel, A., Kritas, S.K., Papageorgiou, D.K., 2009. Occurrence, virulence genes and antibiotic resistance of

Please cite this article as: Farrokh, C., et al., Review of Shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy production, International Journal of Food Microbiology (2012), http://dx.doi.org/10.1016/j.ijfoodmicro.2012.08.008

Escherichia coli O157 isolated from raw bovine, caprine and ovine milk in Greece. Food Microbiology 26, 865–871.
Son, I., Van Kessel, J.A., Karns, J., 2009. Genotypic diversity of *Escherichia coli* in a dairy farm. Foodborne Pathogens and Disease 6, 837–847.
Spano, G., Goffredo, E., Beneduce, L., Tarantino, D., Dupuy, A., Massa, S., 2003. Fate of *Escherichia coli* O157:H7 during the manufacture of Mozzarella cheese. Letters in Applied Microbiology 36, 73–76.
Sperber, W.H., 1983. Influence of water activity on foodborne bacteria — a review. Journal of Food Protection 46, 142–145.
Stefan, A., Scaramagli, S., Bergami, R., Mazzini, C., Barbanera, M., Perelle, S., Fach, P., 2007. Real-time PCR and enzyme-linked fluorescent assay methods for detecting Shiga-toxin-producing *Escherichia coli* in mincemeat samples. Canadian Journal of Microbiology 53, 337–342.
Stephan, R., Buehler, K., 2001. A survey of the prevalence of *Escherichia coli* O157 and other Shiga toxin-producing *Escherichia coli* in bulk-tank milk samples from north-east Switzerland. Archiv für Lebensmittelhygiene 52, 122–123.
Stephan, R., Kuhn, K., 1999. Prevalence of verotoxin-producing *Escherichia coli* (VTEC) in bovine coli mastitis and their antibiotic resistance patterns. Zentralblatt für Veterinarmedizin B 46, 423–427.
Stephan, R., Schumacher, S., Corti, S., Krause, G., Danuser, J., Beutin, L., 2008. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* in Swiss raw milk cheeses collected at producer level. Journal of Dairy Science 91, 2561–2565.
Strachan, N.J.C., Doyle, M.P., Kasuga, F., Rotariu, O., Ogden, I.D., 2005. Dose response modelling of *Escherichia coli* O157 incorporating data from foodborne and environmental outbreaks. International Journal of Food Microbiology 103, 35–47.
Strachan, N.J.C., Dunn, G.M., Mary, E., Locking, M.E., Reid, T.M.S., Ogden, I.D., 2006. *Escherichia coli* O157: burger bug or environmental pathogen? International Journal of Food Microbiology 112, 129–137.
Strawn, L.K., Danyluk, M.D., 2010. Fate of *Escherichia coli* O157:H7 and *Salmonella* spp. on fresh and frozen cut mangoes and papayas. International Journal of Food Microbiology 138, 78–84.
Synge, B.A., Chase-Topping, M.E., Hopkins, G.F., McKendrick, I.J., Thomson-Carter, F., Gray, D., Rusbridge, S.M., Munro, F.I., Foster, G., Gunn, G.J., 2003. Factors influencing the shedding of verocytotoxin-producing *Escherichia coli* O157 by beef suckler cows. Epidemiology and Infection 130, 301–312.
Tchaptchet, S., Hansen, J., 2011. The Yin and Yang of host-commensal mutualism. Gut Microbes 2, 347–352.
Thomas, K.M., McCann, M.S., Collery, M.M., Logan, A., Whyte, P., McDowell, D., Duffy, G., 2011. Tracking verocytotoxigenic *Escherichia coli* O157, O26, O111, O103 and O145 in Irish cattle. International Journal of Food Microbiology 153, 288–296.
Thomson, D.U., Loneragan, G.H., Thornton, A.B., Lechtenberg, K.F., Emery, D.A., Burkhardt, D.T., Nagaraja, T.G., 2009. Use of a siderophore receptor and porin proteins-based vaccine to control the burden of *Escherichia coli* O157:H7 in feedlot cattle. Foodborne Pathogens and Disease 6, 871–877.
Thornton, A.B., Thomson, D.U., Loneragan, G.H., Fox, J.T., Burkhardt, D.T., Emery, D.A., Nagaraja, T.G., 2009. Effects of a siderophore receptor and porin proteins-based vaccination on fecal shedding of *Escherichia coli* O157:H7 in experimentally inoculated cattle. Journal of Food Protection 72, 866–869.
Tobe, T., Beatson, S.A., Taniguchi, H., Abe, H., Bailey, C.M., Fivian, A., Younis, R., Matthews, S., Marches, O., Frankel, G., Hayashi, T., Pallen, M.J., 2006. An extensive repertoire of type III secretion effectors in *Escherichia coli* O157 and the role of lambdoid phages in their dissemination. Proceedings of the National Academy of Sciences of the United States of America 103, 14941–14946.
Tzschoppe, M., Martin, A., Beutin, L., 2012. A rapid procedure for the detection and isolation of enterohaemorrhagic *Escherichia coli* (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104:H4 strain from ready-to-eat vegetables. International Journal of Food Microbiology 152, 19–30.
Upton, P., Coia, J.E., 1994. Outbreak of *Escherichia coli* 0157 infection associated with pasteurised milk supply. The Lancet 344, 1015.
Usajewicz, I., Nalepa, B., 2006. Survival of *Escherichia coli* O157:H7 in milk exposed to high temperatures and high pressure. Food Technology and Biotechnology 44, 33–39.
Van Donkersgoed, J., Berg, J., Potter, A., Hancock, D., Besser, T., Rice, D., LeJeune, J., Klashinsky, S., 2001. Environmental sources and transmission of *Escherichia coli* O157 in feedlot cattle. Canadian Veterinary Journal 42, 714–720.
Van Kessel, J.A., Karns, J.S., Lombard, J.E., Kopral, C.A., 2011. Prevalence of *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli* virulence factors in bulk tank milk and in-line filters from U.S. dairies. Journal of Food Protection 74, 759–768.
Vernozy-Rozand, C., Montet, M.P., Ray-Gueniot, S., 2002. Présence d'*E. coli* O157:H7 dans l'eau: un problème en santé publique. Revue de Medecine Veterinaire 153, 235–242.
Vernozy-Rozand, C., Montet, M.P., Berardin, M., Bavai, C., Beutin, L., 2005a. Isolation and characterization of Shiga toxin-producing *Escherichia coli* strains from raw milk cheeses in France. Letters in Applied Microbiology 41, 235–241.
Vernozy-Rozand, C., Mazuy-Cruchaudet, C., Bavai, C., Montet, M.P., Bonin, V., Dernburg, A., Richard, Y., 2005b. Growth and survival of *Escherichia coli* O157:H7 during the manufacture and ripening of raw goat milk lactic cheeses. International Journal of Food Microbiology 105, 83–88.
Verstraete, K., De Zutter, L., Messens, W., Herman, L., Heyndrickx, M., De Reu, K., 2010. Effect of the enrichment time and immunomagnetic separation on the detection of Shiga toxin-producing *Escherichia coli* O26, O103, O111, O145 and sorbitol positive O157 from artificially inoculated cattle faeces. Veterinary Microbiology 145, 106–112.
Vidovic, S., Mangalappalli-Illathu, A.K., Korber, D.R., 2011. Prolonged cold stress response of *Escherichia coli* O157 and the role of rpoS. International Journal of Food Microbiology 146, 163–169.
Vimont, A., Vernozy-Rozand, C., Delignette-Muller, M.L., 2006. Isolation of *E. coli* O157: H7 and non-O157 STEC in different matrices: review of the most commonly used enrichment protocols. Letters in Applied Microbiology 42, 102–108.
Vinten, A.J.A., Potts, J., Avery, L., Strachan, N.J.C., 2009. Microbial pollution of water by livestock: approaches to risk assessment and mitigation. Animal 3, 744–752.
Voitoux, E., Lafarge, V., Collette, C., Lombard, B., 2002. Applicability of the draft standard method for the detection of *Escherichia coli* O157 in dairy products. International Journal of Food Microbiology 77, 213–221.
Vosough Ahmadi, B., Frankena, K., Turner, J., Velthuis, A.G., Hogeveen, H., Huirne, R.B., 2007. Effectiveness of simulated interventions in reducing the estimated prevalence of *E. coli* O157:H7 in lactating cows in dairy herds. Veterinary Research 38, 755–771.
Wahi, S., Bansal, S., Ghosh, M., Ganguli, A., 2006. Growth and survival of *Escherichia coli* O157:H7 during manufacture and storage of Indian cheese (Paneer). Foodborne Pathogens and Disease 3, 184–189.
Wall, P.G., McDonnell, R.J., Adak, G.K., Cheasty, T., Smith, H.S., Rowe, B., 1996. General outbreaks of verocytotoxin producing *Escherichia coli* O157 in England and Wales from 1992 to 1994. Communicable Diseases Report: CDR Reviews, 6, pp. R26–R33.
Weagant, S.D., Bryant, J.L., Bark, D.H., 1994. Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. Journal of Food Protection 57, 629–631.
Wells, J.G., Shipman, L.D., Greene, K.D., Sowers, E.G., Greene, J.H., Camron, D.N., Downes, F.P., Martin, M.L., Griffin, P.M., Ostroff, S.M., 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like toxin producing *Escherichia coli* from dairy cattle. Journal of Clinical Microbiology 29, 985–989.
Wendel, A.M., Johnson, D.H., Sharapov, U., Grant, J., Archer, J.R., Monson, T., Koschmann, C., Davis, J.P., 2009. Multistate outbreak of *Escherichia coli* O157:H7 infection associated with consumption of packaged spinach, August–September 2006: the Wisconsin investigation. Clinical Infectious Diseases 48, 1079–1086.
Wenz, J.R., Barrington, G.M., Garry, F.B., Ellis, R.P., Magnuson, R.J., 2006. *Escherichia coli* isolates' serotypes, genotypes, and virulence genes and clinical coliform mastitis severity. Journal of Dairy Science 89, 3408–3412.
Wetzel, A.N., LeJeune, J.T., 2006. Clonal dissemination of *Escherichia coli* O157:H7 subtypes among dairy farms in Northeast Ohio. Applied and Environmental Microbiology 72, 2621–2626.
Widiashi, D.A., Ido, N., Omoe, K., Sugii, S., Shinagawa, K., 2004. Duration and magnitude of faecal shedding of Shiga toxin-producing *Escherichia coli* from naturally infected cattle. Epidemiology and Infection 132, 67–75.
Williams, A.P., Avery, L.M., Killham, K., Jones, D.L., 2005. Persistence of *Escherichia coli* O157 on farm surfaces under different environmental conditions. Journal of Applied Microbiology 98, 1075–1083.
Wilson, J.B., McEwen, S.A., Clarke, R.C., Leslie, K.E., Walter-Toews, D., Gyles, C.L., 1993. Risks factors for bovine infection with verocytotoxigenic *Escherichia coli* in Ontario, Canada. Preventive Veterinary Medicine 16, 159–170.
Wood, J.C., McKendrick, I.J., Gettinby, G., 2007. A simulation model to assess herd-level intervention strategies against *E. coli* O157. Epidemiology and Infection 135, 749–764.
Zadik, P.M., Chapman, P.A., Siddons, C.A., 1993. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. Journal of Medical Microbiology 39, 155–158.
Zdragas, A., Samouris, G., Filiousis, G., Petridou, E., Giantzi, V., 2009. Isolation of *Escherichia coli* O157:H7 from raw ewe's and goat's milk in Greece. Archiv für Lebensmittelhygiene 60, 36–38.
Ziebell, K., Konczy, P., Yong, I., Frost, S., Mascarenhas, M., Kropinski, A.M., Whittam, T.S., Read, S.C., Karmali, M.A., 2008. Applicability of phylogenetic methods for characterizing the public health significance of verocytotoxin-producing *Escherichia coli* strains. Applied and Environmental Microbiology 74, 1671–1675.
Zschöck, M., Hamann, H.P., Kloppert, B., Wolter, W., 2000. Shiga-toxin producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properties. Letters in Applied Microbiology 31, 203–208.
Zweifel, C., Rusch, M., Corti, S., Stephan, R., 2006. Determination of various microbiological parameters in raw milk and raw milk cheese produced by bio-farms. Archiv für Lebensmittelhygiene 57, 13–16.
Zweifel, C., Giezendanner, N., Corti, S., Krause, G., Beutin, L., Danuser, J., Stephan, R., 2010. Characteristics of Shiga toxin-producing *Escherichia coli* isolated from Swiss raw milk cheese within a 3-year monitoring program. Journal of Food Protection 73, 88–91.

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