

## Shiga toxin-producing *Escherichia coli* (STEC)

*Escherichia coli* are bacteria that form part of the normal gut flora of humans and other warm-blooded animals. Although most *E. coli* are considered harmless, certain strains can cause severe illness in humans, particularly Shiga toxin-producing *E. coli* (STEC). Infection with STEC is the main cause of haemolytic uraemic syndrome, a condition which can be fatal in humans.

### Description of the organism

*E. coli* are Gram-negative, rod-shaped bacteria and are members of the family *Enterobacteriaceae*. Other species of the genus *Escherichia* include *E. adecarboxylata*, *E. blattae*, *E. fergusonii*, *E. hermanii* and *E. vulneris* (Meng and Schroeder 2007).

Pathogenic *E. coli* are classified into specific groups based on the mechanisms by which they cause disease and clinical symptoms. These categories include enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and diffusely adhering *E. coli* (DAEC) (Montville and Matthews 2005). STEC are Shiga-toxin producing *E. coli*, also known as verocytotoxin-producing *E. coli* (VTEC). The STEC strains that cause haemorrhagic colitis (bloody diarrhoea) belong to the EHEC group of pathogenic *E. coli* (Yoon and Hovde 2008). In developed countries EHEC is the most serious of the pathogenic *E. coli*, however, in developing countries EPEC is a major disease causing agent in children (Meng and Schroeder 2007; Ochoa et al. 2008).

Strains of *E. coli* can be characterised serologically based on the detection of specific O (somatic), H (flagella) and K (capsule) antigens. For most *E. coli* strains the O and H antigens are sufficient to identify the strain. For example, *E. coli* O157:H7 is the leading cause of STEC infections internationally (Meng and Schroeder 2007; Gyles 2007).

### Growth and survival characteristics

The growth and survival of *E. coli* depends on a number of environmental factors such as temperature, pH, water activity ( $a_w$ ) and the composition of the food (refer to Table 1).

The temperature range for growth of *E. coli* is 7–8°C to 46°C, with an optimum temperature of 35–40°C (ICMSF 1996). Heat resistance of *E. coli* in food is dependent on the composition, pH and  $a_w$  of the food. The heat resistance of *E. coli* increases as the  $a_w$  decreases. Also, *E. coli* is more resistant to heat when it is in its stationary phase of growth compared to its log phase of growth (Desmarchelier and Fegan 2003). Low temperature has little effect on *E. coli* survival. Strawn and Danyluk (2010) showed that *E. coli* O157:H7 was able to survive on mangoes and papayas stored at -20°C for at least 180 days.

*E. coli* grow in a broad pH range of 4.4–10.0, with an optimum pH of 6–7 (Desmarchelier and Fegan 2003). A study by Molina et al. (2003) demonstrated that STEC are tolerant to acidic conditions, with many STEC strains able to survive at pH 2.5–3.0 for over 4 hours. *E. coli* O91:H21 was able to survive at pH 3.0 for more than 24 hours. Arnold and Kaspar (1995) found that *E. coli* O157:H7 is more tolerant to acid when it is in stationary growth phase or starved during its log-phase of growth. Therefore STEC may be able to survive and grow in food products previously considered too acidic to support the survival of foodborne pathogens. The effect of pH on *E. coli* survival, however, is dependent on the type of acid

present. For example, *E. coli* O157:H7 can survive in a growth medium adjusted to pH 4.5 with hydrochloric acid but not when adjusted to the same pH with lactic acid (ICMSF 1996).

The minimum  $a_w$  required for growth of *E. coli* is 0.95. In sub-optimal temperature or pH conditions, a higher  $a_w$  value is required for growth of *E. coli* (Desmarchelier and Fegan 2003).

*E. coli* are facultative anaerobic organisms so do not require oxygen for growth. However, they grow better in aerobic conditions (Meng and Schroeder 2007).

**Table 1:** Limits for growth of *E. coli* when other conditions are near optimum (ICMSF 1996; Desmarchelier and Fegan 2003)

	Minimum	Optimum	Maximum
Temperature (°C)	7–8	35–40	46
pH	4.4	6–7	10.0
Water activity	0.95	0.995	–

### Symptoms of disease

Infection with STEC can result in no clinical symptoms (asymptomatic infection) or can cause diarrhoea (may progress to bloody diarrhoea), abdominal cramps, vomiting and fever. The onset of illness is 3–8 days (median of 3–4 days). Most patients recover within 10 days of the initial onset of symptoms (Meng and Schroeder 2007; WHO 2011). In some cases, patients develop haemolytic uraemic syndrome (HUS). HUS is characterised by haemolytic anaemia, thrombocytopenia (decrease in blood platelets) and kidney failure. HUS can also have neurological effects and cause seizures, stroke and coma (WHO 2011). Approximately 6.3% of STEC infected individuals develop HUS, with a fatality rate of 4.6%. Children are more susceptible, with 15.3% of children under five years of age developing HUS following STEC infection (Gould et al. 2009).

STEC are shed in the faeces of infected individuals for several weeks. In children the median shedding time is 13 days (range of 2–62 days) for individuals with diarrhoea. In people who develop HUS, the median shedding time is 21 days (range 5–124 days) (Meng and Schroeder 2007; Pennington 2010).

### Virulence and infectivity

STEC strains produce two types of Shiga toxins (Stx1 and Stx2). Stx1 is virtually identical to the toxin produced by *Shigella dysenteriae* serotype 1. The presence of Stx2 is significantly associated with human disease (Spears et al. 2006). Stx are toxic to Vero cells (African green monkey kidney cells) and so are also known as verotoxins (VT). The term STEC is used interchangeably with VTEC. In the laboratory, Vero cells can be used to detect Stx activity, as Stx causes Vero cell death (Desmarchelier and Fegan 2003; Meng and Schroeder 2007).

Due to the acid resistance of STEC, when ingested it is able to survive in the stomach environment and attach to the cells of the intestine. Some STEC strains form a characteristic attaching and effacing lesion on the intestinal cells. The presence of these lesions is a risk factor for the development of HUS (Gyles 2007). Stx produced by STEC is able to bind to specific receptors on susceptible host cells, resulting in the death of these cells. Vascular

endothelial cells are a primary target for Stx. Hence production of sufficient Stx results in damage to the blood vessels in the colon and subsequent bloody diarrhoea. If sufficient Stx is taken up by the blood and circulated through the body, this can lead to impaired kidney and neurological function and the development of HUS (Desmarchelier and Fegan 2003; Gyles 2007).

### Mode of transmission

STEC are transmitted by the faecal-oral route by either consumption of contaminated food or water, from direct contact with infected animals or via person-to-person contact. It is estimated that 85% of STEC infections are transmitted by food (Meng and Schroeder 2007; Gyles 2007).

### Incidence of illness and outbreak data

Infection with STEC is a notifiable disease in all Australian states and territories. The incidence of STEC infections notified in Australia in 2012 was 0.5 cases per 100,000 population (112 cases), which includes both foodborne and non-foodborne cases. This is the same as the previous 5 year mean of 0.5 cases per 100,000 population per year (ranging from 0.4–0.6 cases per 100,000 population per year) (NNDSS 2013). *E. coli* O157 was the most common STEC identified in Australia in 2010 (58.8% of cases), the next most common was *E. coli* O111. There was 1 case of STEC-associated HUS reported in Australia in 2010 (OzFoodNet 2012). Notified cases of STEC infection are influenced by different jurisdictional practices. South Australia routinely tests all bloody stools for STEC via PCR and subsequently they have the highest notification rate in the country (2.8 cases per 100,000 population compared to 0.0–1.4 cases per 100,000 population for the other jurisdictions in 2012) (OzFoodNet 2012; NNDSS 2013).

The notification rate for STEC in New Zealand in 2011 was 3.5 cases per 100,000 population (154 cases). This was a slight increase from the 2010 rate of 3.2 cases per 100,000 population (Lim et al. 2012).

In the United States (US) the notification rate for STEC in 2010 was 1.78 cases per 100,000 population. This was a slight increase from the 2009 rate of 1.53 cases per 100,000 population (CDC 2012). In the European Union there were 1.93 cases of STEC infection per 100,000 population in 2011 (ranging from 0–6.80 cases per 100,000 population between countries). This was a 159.4% increase in the number of cases from 2010 due to the *E. coli* O104:H4 outbreak that affected nearly 4,000 people (EFSA 2013).

The incidence of STEC infections has a seasonal association, with the number of cases increasing during the warmer months. In Australia STEC is most prevalent from November to April (OzFoodNet 2010).

Foods associated with outbreaks of STEC include undercooked ground beef, fresh produce, unpasteurised juices, salami, cheese and raw (unpasteurised) milk (Yoon and Hovde 2008; FDA 2012) (refer to Table 2).

**Table 2:** Selected major foodborne outbreaks associated with STEC (>50 cases and/or ≥1 fatality)

Year	Strain	Total no. cases (fatalities)	Food	Country	Comments	References
2011	O104:H4	3842 (53)	Fenugreek sprouts	Germany	Imported fenugreek seeds likely source of STEC contamination. Fenugreek sprout related STEC illness in France linked to outbreak (same sprout seeds)	(EFSA 2011; Robert Koch Institut 2011)
2009	O157:H7	80	Raw pre-packaged cookie dough	US	STEC isolated from sample of cookie dough at the factory, however, it was different to the outbreak strain	(CDC 2009)
2006	O157:H7	205 (3)	Pre-packaged spinach	US	STEC isolated from river, cattle and wild pig faeces near spinach field	(California Food Emergency Response Team 2007)
1996-1997	O157:H7	490 (20)	Cooked meat products	Scotland	Either inadequate cooking or cross-contamination from raw meat to cooked products	(Bell and Kyriakides 1998)
1996	O157:H7	7966 (3)	White radish sprouts	Japan	Suspected product supplied in school meals	(Montville and Matthews 2005)
1995	O111:H7	161 (1)	Uncooked fermented mettwurst	Australia	No starter culture used, pH drop during fermentation and water activity during drying not monitored. Product released before maturation was completed	(Chivell 1995)
1993	O157:H7	731 (4)	Hamburgers	US	Insufficient cooking of hamburgers	(Meng et al. 2007)

## Occurrence in food

The major animal reservoir of STEC is ruminants, in particular cattle and sheep (Gyles 2007). Individual animals can carry more than one serotype of STEC (Barlow and Mellor 2010). Meat derived from these animals may become contaminated with STEC organisms if the meat is exposed to faecal material during processing. A study of faecal samples from Australian beef cattle showed 10% of samples (n=300) were STEC positive, with *E. coli* O157 isolated from 1.7% of all samples (Barlow and Mellor 2010). Barlow et al. (2006) isolated STEC from 16% of ground beef (n=285) and 40% of lamb cuts (n=275) sampled in Australia, although the serotypes isolated were not associated with reported human cases in Australia. The detection of STEC at a substantially higher rate in lamb is consistent with the higher concentration and prevalence of *E. coli* on sheep carcasses compared to beef carcasses (Phillips et al. 2001a; Phillips et al. 2001b). The reported prevalence of STEC in bulk tank milk internationally is 0–33.5% (FSANZ 2009).

STEC outbreaks have occurred due to the consumption of fruits and vegetables. Fresh produce may be contaminated due to irrigation with contaminated water or the use of soil treated with farm effluent (Fremaux et al. 2008). The presence of STEC on seafood and poultry at retail may be due to cross-contamination or harvesting seafood from contaminated waters (Desmarchelier and Fegan 2003). STEC has been found to survive for months in soil and manure. It can survive for long periods of time in water and has been isolated from ponds, streams, wells and water troughs. Waterborne transmission of STEC has been reported, both from contaminated drinking water and from recreational water (e.g. swimming) (Fremaux et al. 2008; WHO 2011).

## Host factors that influence disease

People of all ages are susceptible to infection with STEC. However, the young and the elderly are more susceptible and are more likely to develop more serious symptoms (FDA 2012).

## Dose response

The dose response relationship for STEC is complicated by the number of serotypes and the association of STEC with a variety of foods. The infective dose of *E. coli* O157:H7 is estimated to be very low, in the range of 10–100 cells. The infective dose of other STEC serotypes is suspected to be slightly higher (FDA 2012).

Dose response models have been developed for *E. coli* O157:H7. Teunis et al. (2004) used data from an *E. coli* O157:H7 outbreak at a school in Japan to estimate the dose required to cause disease. In children the estimated ingested dose was 31 organisms, with 25% of exposed children becoming ill. In adults the estimated ingested dose was 35 organisms, with 16% of exposed adults becoming ill.

Haas et al. (2000) used data from a prior animal study undertaken by Pai et al. (1986) and validated their model by comparison with two human outbreaks, one foodborne and the other waterborne, that occurred in the US. This model estimated that the dose required for 50% of the exposed population to become ill was  $5.9 \times 10^5$  organisms. The corresponding probability of illness for the ingestion of 100 organisms was  $2.6 \times 10^{-4}$ .

Human feeding trial data has been used to generate a dose response model for *E. coli* serotypes other than *E. coli* O157:H7 (*E. coli* O111 and O55) (Haas et al. 2000). The model estimated the dose required for 50% of the exposed population to become ill was  $2.55 \times 10^6$  and the probability of illness for ingestion of 100 organisms was  $3.5 \times 10^{-4}$ .

### Recommended reading and useful links

Desmarchelier PM, Fegan N (2003) Enteropathogenic *Escherichia coli*. Ch 9 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p. 267-310

Duffy G (2006) Emerging pathogenic *E. coli*. Ch 10 In: Motarjemi Y, Adams M (eds) Emerging Foodborne Pathogens. Woodhead Publishing, Cambridge, p. 253-281

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 74–78.  
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EFSA (2011) Tracing seeds, in particular fenugreek (*Trigonella foenum-graecum*) seeds, in relation to the Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Germany and France.

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EFSA (2013) The European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2011. EFSA Journal 11(4):3129

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<http://www.fda.gov/Food/FoodbornellnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm>. Accessed 27 March 2013

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