



# Enterohaemorrhagic *E. coli* (EHEC)

Family of *Enterobacteriaceae*  
Genus *Escherichia*  
Bacterium

## Characteristics and sources of Enterohaemorrhagic *E. coli* (EHEC)

### Main microbiological characteristics

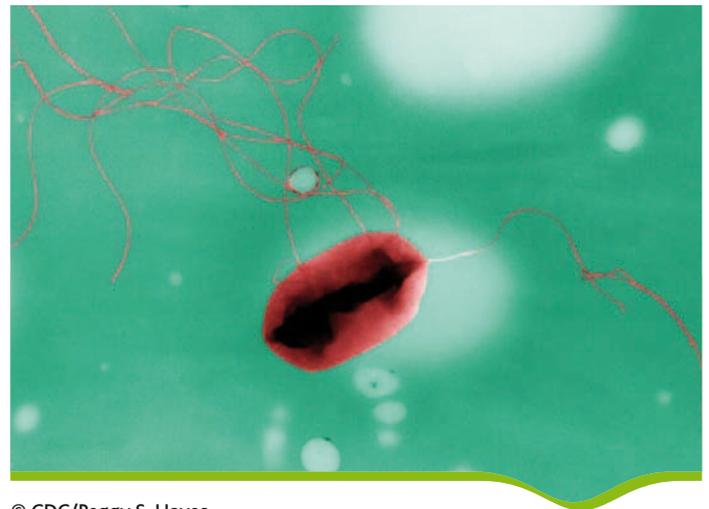
*Escherichia coli* (*E. coli*) is a Gram-negative, oxidase negative bacillus that can live in aerobic and anaerobic environments, with a length of 2 to 4  $\mu\text{m}$  and a diameter of around 0.6  $\mu\text{m}$ . While *E. coli* is a bacterium normally found in the digestive microflora of humans and warm-blooded animals, some *E. coli* strains are pathogenic because they have acquired virulence factors. On the basis of the clinical signs observed in patients, pathogenic *E. coli* strains are grouped into pathovars (or pathotypes), which include enterohaemorrhagic *E. coli* (EHEC).

In humans, EHEC strains can cause various illnesses ranging from benign watery diarrhoea to haemorrhagic colitis which may evolve into severe forms such as Haemolytic Uraemic Syndrome (HUS), primarily in young children, and Thrombotic Microangiopathy (TMA) in adults.

EHEC releases toxins called shiga toxins (also called verotoxins), which cause damage to the vascular endothelium, mainly in the intestines, kidneys and brain. Shiga toxins Stx1 and Stx2 are encoded by *stx* genes. All *E. coli* strains possessing a *stx* gene are called Shiga Toxin-producing *E. coli* (STEC) or Verotoxin-producing *E. coli* (VTEC).

EHEC strains belonging to numerous different *E. coli* serotypes, characterised by their somatic O antigen and their flagellar H antigen, have been involved in episodes of haemorrhagic colitis and HUS. *E. coli* O157:H7 was the first identified serotype and is currently the most frequently isolated in patients.

'Typical' EHEC strains cause so-called 'attaching and effacing' lesions on the mucosal cells of the distal ileum and colon, particularly through the role of a membrane protein, intimin. This protein is encoded by the *eae* gene, which is part of the chromosomal locus of enterocyte effacement (LEE). The strains that have most frequently been involved in outbreaks have been defined by ANSES as 'major typical EHEC' strains. They belong to serotypes O26:H11, O103:H2, O111:H8, O145:H28 and O157:H7 and their non-motile derivatives. Furthermore, Karmali and his team classified STEC serotypes into five seropathotypes (from A to E) according to their relative incidence in human infections, their outbreak frequency, and their link, when applicable, to severe clinical symptoms.



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'Atypical' EHEC strains are strains that do not have the *eae* gene and therefore do not cause attaching and effacing lesions. These strains have other mechanisms of attachment to the intestinal mucosa. Numerous adhesins have been described but their actual role in the pathogeny of these strains remains to be clarified. This is the case of the strains in serogroups O91 and O104, including strain O104:H4, which caused two outbreaks in Germany and France in 2011.

Table 1 gives growth characteristics for the majority of *E. coli* O157:H7 strains, the most commonly studied serotype.

Table 1. Growth characteristics of *E. coli* O157:H7

Parameters	Growth	
	Optimum	Extremes
Temperature ( $^{\circ}\text{C}$ )	40	6 45.5
pH	6-7	4.4 9
$a_w$	0.995	0.95
NaCl (%)	0	8.5

## Hazard sources

The digestive tract of domestic ruminants, and more particularly cattle, is the main reservoir of STEC. As healthy carriers, they contribute to the contamination of the environment with the bacteria found in their faeces. To a lesser extent, other farm animals and wild animals, including some game animals, can also be healthy carriers of STEC. Studies undertaken in cattle show that depending on the herd, 20 to 80% of animals can be carriers of STEC (detection of *stx* genes in faecal matter), but *E. coli* O157:H7 is isolated in few animals (0 to 3%).

The persistence of STEC strains on farms is due to digestive carriage by the animals and contamination from contact between animals, but also to contamination of the soil (meadows, fields) and surface water from contaminated animal dung or fertiliser (manure, slurry) used on farmland. Animal feed (grass, fodder) and drinking water may thus be contaminated. STEC can survive for several weeks in the farm environment (such as in water trough sediments, faeces and manure on the soil).

Various plants consumed by humans can be contaminated with STEC, either through manure from contaminated animals, or when contaminated water is used for irrigation.

## Transmission routes

Since they can be directly and indirectly transmitted from animal reservoirs to humans, these bacteria should be considered as zoonotic agents. Direct transmission is possible through contact with infected animals or their excrement, as well as from person to person (faecal-oral inter-human transmission). The main route of transmission is indirect through the consumption of foods of animal or plant origin and drinking water that has been contaminated by a soiled environment, most often by the faecal matter of infected animals. In the United States, epidemiological studies show that consumption of contaminated foods, person-to-person transmission, ingestion of contaminated water and contact with animals (mainly cattle) respectively account for 66%, 20%, 12% and 2% of cases of contamination.

### Recommendations for primary production (farm, crops)

- Strict compliance with general hygiene rules with limitation of faecal contamination during the primary production of foodstuffs is an essential pre-requisite.
- Compliance with good crop-growing practices, particularly for plants that are to be consumed raw or are intended for the production of seeds for sprouting (compliance with regulations on the spreading of fertilising materials, control of the bacteriological quality of irrigation water) is essential to prevent contamination.
- Protecting drinking-water sources and aquifers, including those that directly serve the food processing industry, against contamination with animal waste, is essential.

## Human foodborne illness

### Nature of the disease

Characteristics of the disease are summarised in [Table 2](#).

**Susceptible population groups<sup>(1)</sup>:** Young children (especially under the age of 3 years) and elderly people for typical EHEC.

### Dose-effect<sup>(2)</sup> and dose-response<sup>(3)</sup> relationships

The amount of ingested bacteria causing the disease with high probability is low. During the 2005 French outbreak that involved deep-frozen ground beef, the average concentration of *E. coli* O157:H7 in the implicated ground beef was six per gram. The dose that caused HUS in half of the exposed individuals was estimated at 600 bacteria for children under the age of 5 years and 3,000 bacteria for children aged 6 to 10 years. The latter is equivalent to the dose that was estimated for children in the same age group during the outbreak caused by the consumption of radish sprouts in Japan in 1996, whereas the dose was evaluated at around 10<sup>6</sup> bacteria for adults.

### Epidemiology

In France, surveillance is focused on HUS in children under the age of 15 years. It is coordinated by the French Institute for Public Health Surveillance (InVS).

From 1996 to 2009, the annual incidence ranged from 0.59 to 1.01 cases/100,000 (average for the period: 0.74). Nearly all of these HUS cases were sporadic forms, with a resurgence in the summer. The incidence was highest in very young children. Since 1996, the highest average annual incidence has been encountered in the Franche-Comté and Brittany regions. Serogroup O157 (83% of cases) has been predominant in these confirmed cases of EHEC infection (64% in Europe from 2002 to 2006). Several non-O157 serogroups have also been detected: O26 (6%), O103 (3%), O145 (2%), O91 (1%), O111 (1%) and O55 (1%). The proportion of non-O157 serogroups was 10% from 1996 to 2001 and 26% from 2002 to 2008.

The number of cases of infection with these strains has mostly likely been underestimated due to a lack of effective isolation strategies for non-O157 EHEC.

In France, four EHEC outbreaks have been detected and investigated: two *E. coli* O157:H7 outbreaks linked to the consumption of deep-frozen ground beef, which occurred in 2005 and 2011; one *E. coli* O26 outbreak linked to the consumption of raw milk cheeses, which occurred in 2005; and one outbreak due to an atypical O104:H4 strain linked to the consumption of sprouts consumed raw in 2011.

(1) Susceptible population group: people with a higher than average probability of developing symptoms of the disease, or serious forms of the disease, after exposure to a foodborne hazard [definition used for ANSES datasheets].

(2) The relationship between the dose (the amount of microbial cells ingested during a meal) and the effect on an individual

(3) For a given effect, the relationship between the dose and the response, i.e. the probability of this effect appearing in the population.

Table 2. Disease characteristics

Mean incubation period	Target population	Main symptoms	Duration of symptoms	Duration of the contagious period	Complications	Asymptomatic forms
3-4 days (varies from 2 to 12 days)	Main symptoms	Banal diarrhoea or Haemorrhagic colitis: abdominal cramps and initially watery then bloody diarrhoea in a generally afebrile or subfebrile patient	5 to 12 days	At least one week in adults, but can be longer in children	Haemolytic Uremic Syndrome (HUS) in 5 to 8% of cases. Lethality from HUS in children under the age of 15 years is 1% in France Thrombotic Microangiopathy (TMA) (lethality in elderly people: 50%) Severe neurological complications can appear in 25% of HUS cases Chronic kidney failure in 50% of HUS survivors	Humans can be EHEC carriers without showing clinical signs

# Role of food

## Main foods to consider

The main foods involved in EHEC outbreaks around the world have been: inadequately cooked ground beef, unpasteurised dairy products, raw vegetables (lettuce, white radish sprouts, sprouted seeds) and unpasteurised products of plant origin (apple juice), and drinking water. Note that plants and water have been responsible for outbreaks affecting hundreds of people over the past few years.

The contamination of foods of animal origin with bacteria of faecal origin occurs for example at the slaughterhouse (skinning and evisceration of animals) for meats, or on the farm during milking for milk, particularly when general hygiene rules are not followed. For plants, this contamination can occur when contaminated manure or ruminant farm effluents are spread on the soil where they are grown, or when contaminated irrigation water is used. As for leafy vegetables (lettuces, spinach), the bacterium can penetrate inside the plant tissues, and migrate and persist in the plant without multiplying. Drinking water can be contaminated either accidentally or due to a treatment failure.

## Inactivation treatments in industrial environments (Table 3)

*E. coli* O157:H7 is not considered to be a heat-resistant bacterium. The thermal treatments that are considered effective against *Salmonella* spp. are also effective against *E. coli* O157:H7. It has been shown experimentally that typical strains of EHEC O157:H7, the most commonly studied serotype, can have enhanced survival in acidic conditions (meat or fermented dairy products, fruit juices, dressed salads, etc.) compared to other strains of *E. coli*. No other particular resistance to sanitising treatments has been reported.

## Monitoring in food

STEC comprise 400 serotypes that differ considerably in terms of their physiological characteristics and their pathogenicity for humans. *E. coli* strains of animal, food or environmental origin with the *stx* and *eae* genes are considered to be potentially pathogenic STEC. Moreover, if they belong to serogroups O157, O26, O103, O111 or O145, they are considered to be potentially highly pathogenic STEC since they have the same characteristics as the major typical EHEC strains.

As defined in Amended Regulation (EC) no. 2073/2005<sup>(4)</sup>, surveillance of *E. coli* is the best indicator for the level of process hygiene when monitoring the faecal contamination of a food. To date, the assessments of the European Food Safety Authority (EFSA) have not found there to be a need to define a specific safety criterion for pathogenic STEC. However, these

pathogenic bacteria should be taken into account by professionals when performing hazard analysis and may be screened for in the framework of self-inspections and compliance with the general principles set forth in the 'Hygiene Package'. For the detection of *E. coli* O157 in foodstuffs, a reference method (NF EN ISO 16654<sup>(5)</sup>) and several validated alternative methods are available. For non-O157 strains, an ISO/CEN Draft Technical Specification under validation (ISO TS 13136) is currently used by reference laboratories for the detection of the main potentially highly pathogenic non-O157 STEC serogroups (seropathotype B as proposed by Karmali). Immunomagnetic separation techniques are available for the isolation of strains belonging to these serogroups (O26, O103, O111 and O145).

In France, the Directorate General for Food (DGAL) organises yearly food monitoring and surveillance plans (meat intended for grinding, ground meat, raw milk cheeses). There is currently no harmonised surveillance system at the European level: in an Opinion published on 18 October 2007, EFSA recommended that monitoring should initially concentrate on *E. coli* O157:H7 since this serotype is most frequently associated with severe human infections (including HUS). Monitoring should then be extended to serotypes O26, O91, O103, O111, O145 and other serotypes that are identified by epidemiological data.

### Recommendations to operators

- It is important to underline that the implementation of microbiological analyses for the detection of pathogenic STEC in foods can reduce the risk of clustered HUS cases in children under the age of 15, but it alone is not sufficient.
- Strict compliance with good hygiene practices with limitation of faecal contamination during animal slaughtering and milking and the processing of foodstuffs is an essential prerequisite.
- In France, the Inter-Ministerial Memo DGAL/SDSSA/O2007-8001 of 13 February 2007 on recommendations for the cooking of ground beef to prevent *E. coli* O157:H7 infection for collective catering professionals recommends cooking to obtain an internal temperature of 65°C. Furthermore, a higher internal temperature (70°C) is often recommended in order to combat not only potentially pathogenic STEC, but also other sources of microbial contamination.

(4) Regulation (EC) no. 2073/2005, amended by Regulation no. 1441/2007, on microbiological criteria for foodstuffs, defines two types of microbiological criteria.

(5) Microbiology of foods – Horizontal method for the detection of *Escherichia coli* O157 (July 2001).

Table 3. Inactivation treatments of *E. coli* O157:H7 in industrial environments

Disinfectants		Effects of temperature
<p>Susceptible to all disinfectants authorised in the food industry, as long as recommendations for use are followed.</p> <p>Chemical disinfection treatments for drinking water are effective against these bacteria.</p> <p>Treatment with sodium hypochlorite solutions:</p> <ul style="list-style-type: none"> <li>• lettuce: 20 ppm active chlorine for 2 min. → less than one log reduction.</li> </ul> <p>Ozone treatments:</p> <ul style="list-style-type: none"> <li>• blueberries: 1.7 mg/L water → 1.3 log reduction;</li> <li>• apples: 22 mg/L water → 2.6 log reductions.</li> </ul> <p>For plants, the effectiveness of biocides (ozone, chlorine, etc.) is limited to their surface (biocides have no effect on the bacteria found inside the tissues).</p>		<p>D-values* and z-values**: D<sub>60°C</sub> = 0.5 to 3 min. and z = 3.5 to 7°C</p> <p>NB: the fat content of meat products increases heat-resistance.</p>
Irradiation	UV (253.7 nm)	High Pressures
<p>Beef: 2 kGy → 5 log reductions</p> <p>Lettuce: 1.5 kGy → 4 log reductions</p> <p>Spinach: 1.5 kGy → 3 log reductions</p>	<p>Lettuce: 24 mJ/cm<sup>2</sup> → 2.8 log reductions</p>	<p>Alfalfa seeds: 650 MPa for 15 min. at 20°C → around 5 log reductions</p> <p>Salami: 600 MPa for 3 min. → around 4 log reductions</p>

\* D is the time needed to divide by 10 the initial population of a microbiological hazard.

\*\* z is the variation in temperature (°C) corresponding to a variation by a factor of 10 of the decimal reduction time.

# Domestic hygiene

## Recommendations to consumers

- Personal and collective hygiene remains the basis of prevention. Carefully wash hands after using the toilet, and before preparing and eating meals.
- Thoroughly cook ground meat and products made with ground meat that are to be consumed by young children and elderly people.
- Raw milk and raw milk cheeses should not be consumed by children under the age of 3 years.
- Vegetables, fruits and herbs, particularly those that will be consumed raw, should be thoroughly washed, and then peeled if possible, before they are prepared and consumed.

## References and links

### General references

- AFSSA (2003). Summary of knowledge about Shiga Toxin-producing *Escherichia coli* (STEC).
- AFSSA (2007). Quantitative assessment of the risks associated with *Escherichia coli* O157:H7 in frozen beef burgers consumed at home in France by children under 16 years of age.
- AFSSA (2008). Opinion of 15 July 2008 of the French Food Safety Agency (AFSSA) regarding strains of Shiga Toxin-producing *Escherichia coli* considered pathogenic for humans.
- AFSSA (2010). Opinion of 27 May 2010 of the French Food Safety Agency (AFSSA) on the advisability of revising the definition of pathogenic STEC, specified in AFSSA's Opinion of 15 July 2008.
- ANSES (2011). Opinion of 11 January 2011 of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) on the revised definition of major typical EHEC, the quantitative assessment of risks related to these bacteria in various stages of the food chain, depending on consumption patterns for ground beef, and hazards related to enteropathogenic *E. coli* (EPEC) in food.
- EFSA (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 types. The EFSA Journal (2007) 579: 1-61.

### Useful links

- National Centre of Reference (NCR) for *Escherichia coli* and *Shigella*:
  - Coordinating NCR: research and expert assessment unit for enteric pathogenic bacteria, Institut Pasteur (Paris);
  - Associate laboratory: microbiology department, Hôpital Robert Debré, AP-HP (Paris).
- National Institute for Public Health Surveillance (InVS):
  - [http://www.invs.sante.fr/publications/2006/enquete\\_e\\_coli\\_2003/index.html](http://www.invs.sante.fr/publications/2006/enquete_e_coli_2003/index.html)
  - <http://www.invs.sante.fr/surveillance/shu/index.htm>
- Research laboratory for pathogenic microorganisms in food/National reference laboratory for STEC (STEC NRL): VetAgroSup, Campus vétérinaire de Lyon (Marcy-l'Étoile).
- The European Union Reference Laboratory for *Escherichia coli*, including Verotoxigenic *E. coli* (VTEC): Istituto Superiore di Sanità (ISS) I-00161 (Rome – Italy).