AGENCE FRANÇAISE
DE SÉCURITÉ SANITAIRE
DES ALIMENTS

**AFSSA – Request No. 2010-SA-0031**
**Related Request No. 2008-SA-0122**

Maisons-Alfort, 27 May 2010

# OPINION

**of the French Food Safety Agency**
**on the advisability of revising the definition of pathogenic STEC, specified in AFSSA's Opinion of 15 July 2008.**

THE DIRECTOR GENERAL

## 1. OVERVIEW OF THE REQUEST

On 11 January 2010 the Directorate General for Health (DGS) and the Directorate General for Food (DGAL) asked the French Food Safety Agency (AFSSA) for an opinion on the advisability of revising the definition of pathogenic STEC[1], specified in AFSSA's Opinion of 15 July 2008, according to the scientific data acquired in France or in other countries since 2008.

## 2. BACKGROUND

A home-based foodborne illness outbreak (TIAC) that resulted in a case of haemolytic uraemic syndrome (HUS) and one concomitant case of diarrhoea occurred in early 2009. Frozen beef burgers consumed while not thoroughly cooked, were implicated. The microbiological analysis of a still uneaten beef burger from the same package revealed the presence of *E. coli* O123:H2 (*stx2+, eae+)*, a serotype identified for the first time by the French surveillance system.

The definition of pathogenic STEC is still provisional, as stated in AFSSA's Opinion of 15 July 2008 and repeated in the solicited request. It should be possible to update this definition, as necessary, in light of new clinical observations and epidemiological research data.

The objective of this opinion is thus to provide some answers about this *E. coli* serotype that is rarely identified in human cases, and then broaden the discussion to include scientific knowledge acquired since the publication of AFSSA's definition of pathogenic STEC in 2008, for possible revision.

## 3. ORGANISATION OF THE EXPERT APPRAISAL

The collective expert appraisal of the case was conducted by the Expert Committee on Microbiology (CES) on 11 May 2010, on the basis of an initial report prepared by the Working Group on enterohaemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *Escherichia coli (*EPEC) 2010.

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[1] STEC: Shiga toxin-producing *Escherichia coli*

## 4. DISCUSSION

AFSSA's rationale is based on the Opinion of the CES on Microbiology, which took account of the following:

### 4.1. Review of AFSSA's Opinion of 15 July 2008

AFSSA's Opinion of 15 July 2008 stated:

"To date, pathogenic STEC strains, also referred to in the main text as 'major typical EHEC', may be defined according to the following genetic criteria:

EHEC O157:H7 = *rfbE*$_{O157}$, *fliC*$_{H7}$, *stx*1 and/or *stx*2, *eae*-gamma, (OI#122).

EHEC O26:H11 = *wzx*$_{O26}$, *fliC*$_{H11}$, *stx*1 and/or *stx*2, *eae*-beta, (OI#122).

EHEC O145:H28 = *ihp1*$_{O145}$, *fliC*$_{H28}$, *stx*1 and/or *stx*2, *eae*-gamma, (OI#122).

EHEC O103:H2 = *wzx*$_{O103}$, *fliC*$_{H2}$, *stx*1 and/or *stx*2, *eae*-epsilon, (OI#122).

EHEC O111:H8 = *wbd1*$_{O111}$, *fliC*$_{H8}$, *stx*1 and/or *stx*2, *eae*-theta, (OI#122).

The antigenic formulas are described because membership in a serogroup (O26, for example) does not in itself indicate whether the strains are pathogenic.

It should be emphasised that the proposed definition is no more than a hypothesis which should be revised according to new clinical observations and the results of epidemiological investigations, and to the results of research studies and the development of advanced methods."

Within the context of AFSSA's Opinion of 15 July 2008, the definition of pathogenic STEC strains related only to major typical EHECs. AFSSA has found it necessary to clarify the terms used in this definition in this Opinion.

### 4.2. Criteria for defining human pathogenic *E. coli*

Intestinal pathogenic *E. coli* strains are capable of proliferating, of remaining in the digestive tract by circumventing the host's immune defences, and of damaging gastrointestinal tract cells. Nevertheless, these strains have developed different modes of interaction with their host, resulting in clinical signs of varying severity, which may be accompanied by extra-gastrointestinal complications.

#### 4.2.1. Based on the clinical context: EPEC / EHEC

On the basis of clinical signs observed in patients, in human medicine pathogenic strains of *E. coli* are classified by pathovars, including EPEC and EHEC.

The EPEC pathovar (for "*Enteropathogenic E. Coli*") is responsible for severe diarrhoea, mainly in children under 12 months of age in developing countries (Kaper, Nataro *et al.* 2004; Levine 1987).

The EHEC pathovar (for "*Enterohaemorrhagic E. coli*"), is responsible for various disorders ranging from benign watery diarrhoea to haemorrhagic colitis that can progress to haemolytic uraemic syndrome (HUS) in children and thrombotic microangiopathy (TMA) in adults.

These two distinct pathovars are therefore defined on the basis of quite different human clinical symptoms.

### 4.2.2. Based on the gene pool of the strain

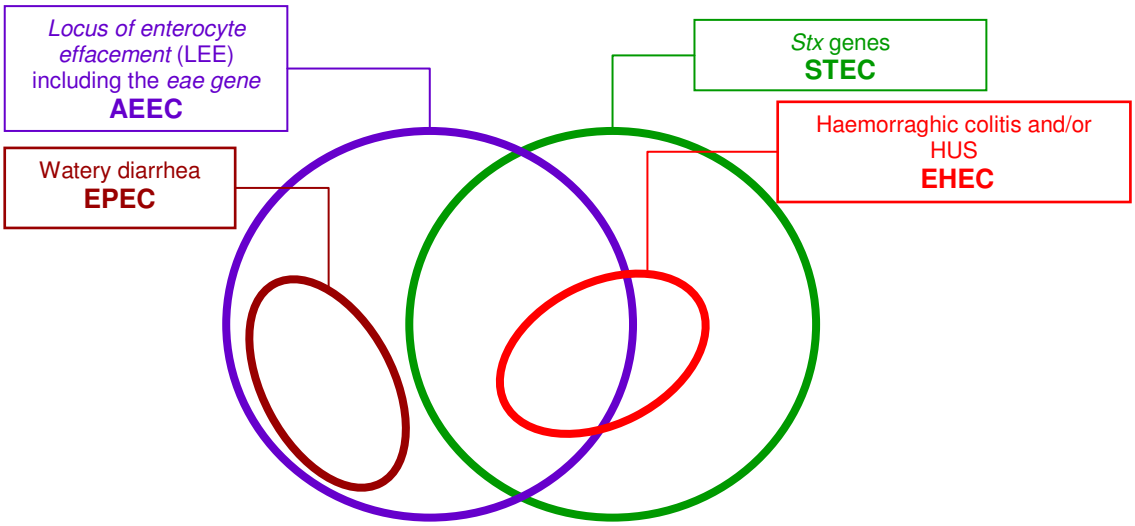
- **AEEC / STEC**

From a histopathological standpoint, the symptoms related to infections due to EPEC are associated with attaching and effacing (A/E) lesions, which are characteristic lesions of intestinal mucosa cells. These A/E lesions are characterised by intimate adherence of the bacteria to enterocytes (Nataro and Kaper 1998). They are due to the combined action of proteins encoded by genes, including the *eae* gene, grouped together in a pathogenicity island, the LEE for "*locus of enterocyte effacement"* (McDaniel, Jarvis *et al.* 1995). All the strains that have the LEE can be classified under the term AEEC for "*Attaching and effacing E. coli*" (Figure 1a). EPEC are the AEEC subgroup responsible for severe diarrhoea in humans, as described above.

*Loci* homologous to LEE have been found in other pathogenic bacteria capable of producing A/E lesions, particularly in certain EHEC (Kaper, Nataro *et al.* 2004).

EHEC are also capable of causing lesions of the vascular endothelium, primarily intestinal, renal and cerebral (O'Loughlin and Robins-Browne 2001). These lesions are due to the action of toxins, the Shiga toxins (Stx), encoded by the *stx* genes carried by bacteriophages. Any *E. coli* strain that has an *stx* gene is termed STEC for "Shiga toxin-producing *E. Coli*". The EHEC form a subgroup of the STECs (Figure 1a) (Caprioli, Morabito *et al.* 2005; Levine 1987).

**Figure 1a: Current classification system of EPEC/EHEC/STEC/AEEC.**




*(See Figure 1c caption)*

- **Typical EHEC / atypical EHEC**

"Typical" EHEC strains have the *eae* gene and cause attaching and effacing (A/E) lesions in the intestinal cells of the distal ileum and colon.

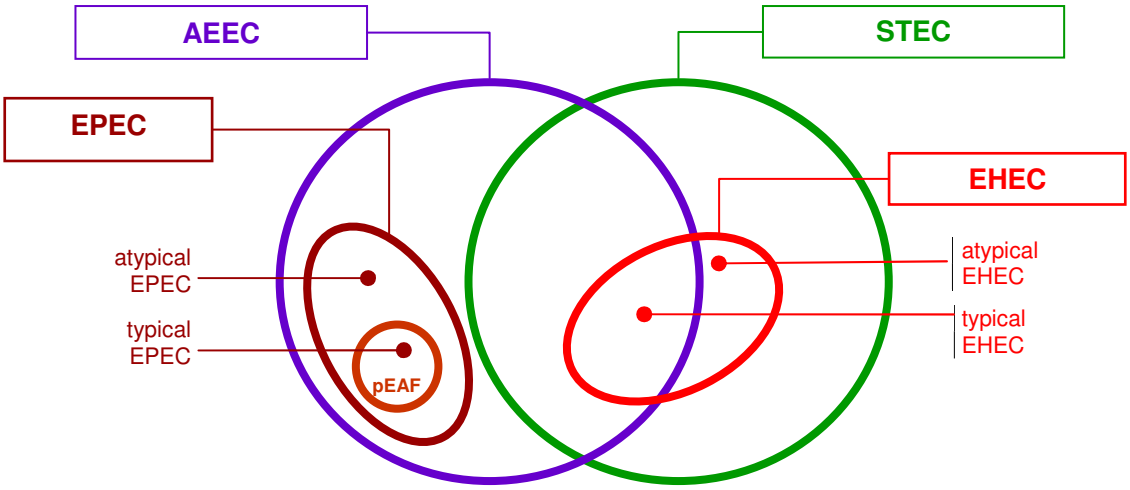
The term "atypical" EHEC is used to designate strains that do not have the *eae* gene and therefore do not produce attaching and effacing lesions (Nataro and Kaper 1998) (Figure 1b). Since colonisation of the digestive tract is a major step in the physiopathology of EHEC, atypical strains have other mechanisms for adhering to the colonic mucosa. Many potential adhesins have been described (see below) but their real involvement in the pathogenesis of these strains still remains unclear.

- **Typical EPECs / atypical EPECs**

"Typical" EPEC strains have a characteristic mode of adherence to cultivated epithelial cells called "localised adherence" (presence of microcolonies, bacterial aggregates, attached to limited zones of the cellular surface). This localised adherence is associated with the presence of an EAF plasmid (*EPEC adherence factor*) encoding in particular for BFP *pili* (*bundle-forming pilus*). BFP *pili* may allow initial adherence of typical EPECs to the cells of the small intestine's mucous membrane.

EPEC strains that do not have the EAF plasmid are referred to as "atypical" (Trabulsi, Keller *et al.* 2002) (Figure 1b). However, other mechanisms of initial adherence still remain to be specified for these strains.

**Figure 1b: Typical and atypical EPEC / typical and atypical EHEC**




*(See Figure 1c caption)*

### 4.2.3. Based on serotypes and their frequency of involvement in human cases: major EHECs

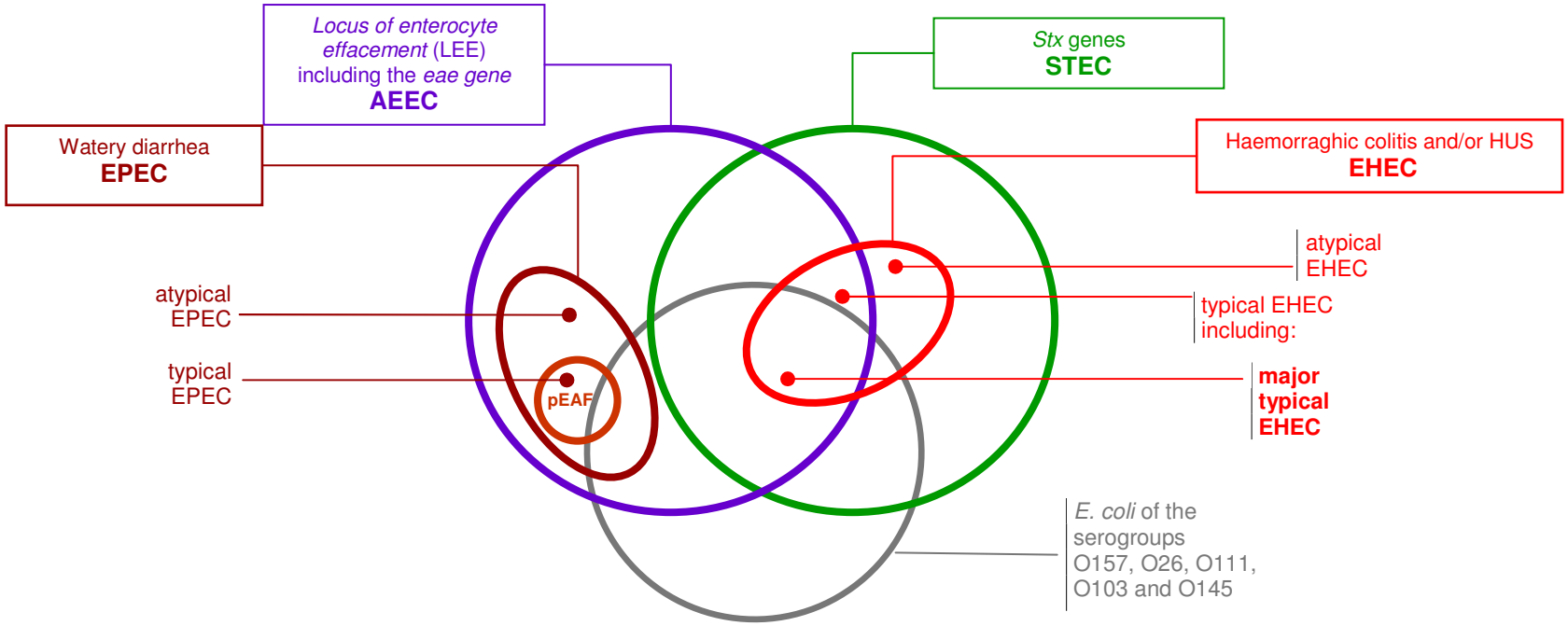
The analysis of worldwide epidemiological data shows that a large number of EHEC serotypes (over 200) are associated with the occurrence of severe clinical symptoms in humans such as haemorrhagic colitis or HUS (Karmali, Mascarenhas *et al.* 2003).

However, in addition to the serotype O157:H7 that is found most frequently [64% of HUS cases in Europe between 2002 and 2006], only a limited number of serotypes has been regularly associated with outbreaks (EFSA 2007).

Therefore, based on data available in 2008, AFSSA defined "major" EHECs, i.e., those most frequently implicated in outbreaks. These strains belong to the serotypes O26:H11, O103:H2, O111:H8, O145:H28 and O157:H7 and their non-motile derivatives (AFSSA 2008) (Figure 1c). These are all typical EHECs.

Serotype O123:H2, which was identified for the first time by the French surveillance system in 2009, when characterising the EHEC strain responsible for a home-based foodborne illness outbreak (TIAC) with a case of HUS and a case of concomitant diarrhoea, is no longer regarded as "major".

**Figure 1c: Venn diagram illustrating the current classification system for EPEC/EHEC/STEC/AEEC based on clinical signs and virulence factors related to strains of *E. coli* [from (Naylor, Gally *et al.* 2005)].** The disks' surfaces and intersections are not proportional to the prevalence of different types of *E. coli.*




**Caption:** All strains having the LEE, including the *eae* gene, irrespective of the related clinical signs, belong to the AEEC group. All strains having genes encoding for Stx toxins, irrespective of the related clinical signs, belong to the STEC group.

EPEC strains are associated with watery diarrhoea in humans and are defined as strains with the LEE and which do not produce Stx toxin. Based on the presence of pEAF plasmid, typical EPEC strains are distinguished from atypical EPEC strains.

EHEC strains are associated with haemorrhagic colitis and/or haemolytic and uraemic syndrome in humans and produce Stx toxins. Most EHECs have LEE and the EHEC LEE+ Stx+ strains are called 'typical EHECs'. Thus, all EHECs are STECs but not all STEC strains, even those that have LEE, are automatically associated with illness in humans.

Typical EHEC strains belonging to serotypes O157:H7, O26:H11, O111:H8, O103:H2 and O145:H28 (and their non-motile derivatives) are the strains most often associated with severe clinical signs (HUS) and with outbreaks, and are called “typical major EHEC” strains.

EPEC: “Enteropathogenic *E. Coli*”; EHEC: “Enterohaemorrhagic *E. Coli*”; STEC: “Shiga toxin-producing *E. Coli”*; AEEC: “Attaching and effacing *E. Coli”*; LEE: “*Locus* of enterocyte effacement”; pEAF: plasmid encoding for EAF factor “EPEC adhesion factor”; *stx*: Genes encoding for Shiga toxins; HUS: haemolytic and uraemic syndrome.

#### 4.2.4.STEC classification according to Karmali *et al.* (2003)

As stated in AFSSA's Opinion of 15 July 2008, Karmali and colleagues classified the STEC serotypes into five seropathotypes (from A to E; Table A) by (i) relative incidence of serotypes in human infections, (ii) their frequency of implication in outbreaks, and (iii) whether or not they are associated with severe clinical symptoms (Karmali, Mascarenhas *et al.* 2003; Konczy, Ziebell *et al.* 2008). Major typical EHECs defined by AFSSA in 2008 belong to the seropathotypes A or B (Table A). Because of its very low incidence in human cases, serotype O123:H2, identified for the first time as part of a home-based foodborne illness outbreak (TIAC) that occurred in early 2009 in France, belongs to seropathotype C.

Table A: Classification of STEC serotypes by seropathotype (Karmali, Mascarenhas *et al.* 2003)

| Seropathotype | Relative incidence | Implication in outbreaks | Association with HUS or haemorrhagic colitis | Serotypes |
|---|---|---|---|---|
| A | High | Frequent | Yes | O157:H7, O157:NM* |
| B | Moderate | Infrequent | Yes | O26:H11, O103:H2, O111:H8/NM, O121:H19, O145:NM |
| C | Low | Rare | Yes | O91:H21, O104:H21, O113:H21 and other serotypes. |
| D | Low | Rare | No | Multiple serotypes |
| E | Non-human** | n.a. | n.a. | Multiple serotypes |

*NM, non-motile.

**STEC serotypes isolated in animals and never associated with human infections.

n.a., not applicable.

## 4.3. Updating of scientific knowledge

The knowledge acquired since the publication of AFSSA's Opinion of 15 July 2008 and derived, firstly, from the study of new EHEC infections described in France and abroad, and secondly, from the results of scientific research, is required in order to judge the advisability of amending the definition of major typical EHECs.

#### 4.3.1. Main serogroups responsible for EHEC infections

- **In France**

Data derived from national surveillance of paediatric HUS

French HUS surveillance data (1996-2006), cited in AFSSA's Opinion of 15 July 2008, revealed the predominance of serogroup O157 (83% of cases) among the HUSs with confirmed EHEC infection. Several non-O157 serogroups were also identified in cases of HUS during this period: O26, O103, O145, O91, O111 and O55. A microbiological or serological confirmation was possible for 75% and 78% of the HUS cases identified through surveillance in 2007 and 2008 respectively.

Table B gives an update of the surveillance data on which the AFSSA Opinion of 15 July 2008 was based. These surveillance data show that serogroup O157 is still mostly isolated, although it was less prevalent in 2007 and 2008 compared with the 1996-2006 period (Table B).

The cumulative proportions of HUS with isolation of an EHEC strain belonging to serogroups O26, O55, O91, O145, O103 and O111 in 2007 and 2008 are respectively 18% and 16% and thus slightly higher than that observed over the 1996-2006 period (14%) (Table B).

As noted in AFSSA's Opinion of 15 July 2008, the proportion of non-O157 serogroups identified in cases of HUS with confirmed EHEC infection had increased in recent years from 10% (1996-2001 period) to 23% (2002-2006 period). By combining the data from 2002 to 2008, this proportion is 26%. This increase may be partially explained by the improvement in identification techniques for non-O157 EHEC serogroups since surveillance began in 1996.

The five serogroups of the major typical EHECs as defined by AFSSA in 2008 were implicated in 95% and 80% of confirmed EHEC:HUS cases for the 1996-2006 and 2007-2008 periods, respectively (Table B).

An EHEC strain was isolated from 23 (31%) and 43 (27%) cases of HUS identified, respectively, in 2007 and 2008. A serogroup or serotype has been established for 17 (74%) of the strains isolated from cases of HUS in 2007 and 37 (86%) in 2008 (Table B). Twenty-six percent of the strains isolated in 2007 and 14% in 2008 thus remained non-serotypable, even after the National Centre of Reference for *E. Coli* and *Shigella* completed molecular typing for some of them.

Serogroup O157 was in the majority among the strains isolated in 2007 and 2008 [43% and 65% respectively (Table C)] as well as for the 1996-2006 period (67%). This serogroup accounted for 58% of serotypable strains in 2007 and 75% in 2008 compared to 77% for the 1996-2006 period. Serogroups not included in the list of major typical EHEC strains defined by AFSSA in 2008 accounted for 23% of the strains isolated and typed in 2007 and 16% in 2008 (Table C). Serotype O121:H19 accounted for 7% of the strains typed during the 2007-2008 period (Table C).

One hundred percent of the EHEC strains isolated in HUS cases in 2007 and 2008 had the *stx2* virulence gene (97% for the period 1996-2006). The proportions of typical EHEC strains isolated having the *eae* virulence gene were stable in 2007 (89%) and 2008 (90%) compared to the 1996-2006 period (93%).

**Table B: The most prevalent EHEC serogroups identified by serology or by culture in paediatric HUS cases in France, 1996-2008 (source: French Institute for Public Health Surveillance [InVS] data)**

| Serogroup | 1996-2006 | 2007 | 2008 |
|---|---|---|---|
| | % | % | % |
| O157 | 83 | 58 | 69 |
| O26 | 6 | 7 | 7 |
| O145 | 2 | 4 | 5 |
| O103 | 3 | 5 | 2 |
| O111 | 1 | 2 | 0 |
| O91 | 1 | 0 | 1 |
| O55 | 1 | 0 | 1 |
| O121 | 0 | 5 | 1 |

**Table C: Serogroups and serotypes of EHEC strains isolated from paediatric HUS cases identified in France in 2007 and 2008.**

| 2007 | | | 2008 | | |
|---|---|---|---|---|---|
| **Serogroup/ Serotype*** | **N** | **%** | **Serogroup/ Serotype*** | **N** | **%** |
| O5:H18 | 1 | 4 | O26 | 5 | 12 |
| O26 | 3 | 13 | O26:H11 | 1 | 2 |
| O121:H19 | 3 | 13 | O101:H33 | 1 | 2 |
| O157:H7 | 1 | 4 | O121:H19 | 1 | 2 |
| O157 | 9 | 39 | O145:H2 | 1 | 2 |
| NST** | 6 | 26 | O157:H7 | 10 | 23 |
| | | | O157 | 18 | 42 |
| | | | NST** | 6 | 14 |
| **Total** | **23** | **100** | **Total** | **43** | **100** |

*The serogroup (or serotype) was obtained through agglutination with the serum panel used by the NCR for *E. coli* and *Shigella* and its affiliated laboratory or determined by molecular typing of the strain at the NCR.

** Non-serotypable according to previously described methods

Data from the NCR for *E. coli* and *Shigella* and its affiliated laboratory

The strains, analysed by the NCR and its affiliated laboratory, are dispatched during a suspected infection with EHEC or sometimes EPEC in a child or an adult. Strains are sent on a voluntary basis and consequently the current NCR strain collection cannot be considered to be representative of all strains involved in human cases. For a better characterisation of EHECs, it would therefore be highly desirable to encourage laboratories to transmit all strains isolated from cases of HUS to the NCR or its affiliated laboratory.

Between 2007 and 2009, the NCR and its affiliated laboratory received strains of *E. coli* taken from 1796 patients for expert appraisal. Among these strains, 231 (12.9%) had at least one *stx* gene (STEC strain). Among these 231 STEC strains, clinical information was available for 191 strains (82.7%) with, for 134 of these cases (70.2%), a HUS (including the adult form: thrombotic micro-angiopathy [TMA]). The distribution of serogroups/serotypes of strains isolated in the 134 cases of HUS is shown in the table in the Annex to this Opinion.

The EHEC strains identified in the cases of HUS mostly had the *eae* gene (79.1%) but 20.9% of strains were atypical EHECs *(eae*-negative) which probably have another adhesion mechanism or partial LEE (Annex).

Forty-four percent of the EHEC strains belonged to serogroup O157, and 14% to serogroup O26 (Annex). The percentage of non-serotypable EHECs remained high (20.1%). These strains do not agglutinate with sera available at the NCR, and the molecular methods used *(rfb-RFLP* for the O antigen and sequencing of the *fliC* gene for the H antigen) showed the emergence of new profiles and new sequences that are more or less similar to those found in the databases, but do not enable a reliable interpretation to be made.

- **In Europe**

Since 2007, European surveillance of EHEC infections has been coordinated by the European Centre for Disease Control. The Member States of the European Union (EU) and certain countries outside the EU (Norway, Iceland and Lichtenstein) transmit data each year on EHEC infections identified in their countries. European data for the years 2006, 2007 and 2008 show a predominance of serogroup O157 in Europe. This serogroup accounted for 48% of confirmed human infections in 2006, 53% in 2007 and 54% in 2008 (EFSA 2009; EFSA 2010). As in France, the most prevalent non-O157 serogroups in Europe in 2006, 2007 and 2008 were O26 (4.5% on average), O103 (2.7% on average), O145, O91 and O111 (each 1% on average). Other non-O157 serogroups were also identified as responsible for human cases at the European level during this period, but with a frequency generally lower than 1% (O44, O86, O113, O117, O119, O124, O128, O146) (EFSA 2009; EFSA 2010).

A major limitation on interpretation of European data is the grouping of strains classified as non-serotyped with strains considered as non-serotypable. This makes it impossible to interpret this category of data, which accounted for respectively 29% and 26% of the data from 2007 and 2008.

Serogroups O157, O26, O103, O111 and O145 accounted for 63% of human EHEC infections identified at the European level during the period 2007-2008 (EFSA 2009; EFSA 2010). In 2008, these five serogroups accounted for 23% of infections identified in Germany, 39% in Austria, 42% in Sweden, 60% in the Netherlands, 69% in Belgium and 99% in the United Kingdom (EFSA 2010). Among these countries, STEC infections are subject to mandatory notification in Germany, Austria, Sweden and the Netherlands (ECDC 2009).

### 4.3.2. Clustered cases of EHEC infections

- **In France**

Since the publication of AFSSA's Opinion of 15 July 2008, 12 episodes of clustered cases of confirmed EHEC infections were identified between 2007 and 2009 by the HUS surveillance system (three in 2007, four in 2008 and five in 2009). These episodes all occurred within a family context, and included either one case of HUS with one or more people presenting symptoms indicative of an EHEC infection in the family circle, or two cases of HUS within the same family.

A strain of EHEC was isolated in nine of the 12 episodes of clustered cases; the isolates had one of the four following profiles: O157:H7 *stx2 eae* (N=6), non-serotypable *stx2 eae* (N=1), O26 *stx2 eae* (N=1), and O123:H2 *stx2 eae* (N=1). Three episodes were confirmed by serology (O157).

A source of contamination was identified for only one of the 12 episodes: the consumption of minced beef that had not been thoroughly cooked for the O123:H2 EHEC foodborne illness outbreak in 2009. The O123:H2 serotype has been only rarely described as a source of clinical infections in the literature. This episode provided the first evidence of this serotype in a paediatric case of HUS notified through surveillance in France. It is also the first description of an episode of clustered cases due to this serotype.

No outbreaks of EHEC have been identified through the surveillance of paediatric HUS cases since the outbreaks which occurred in 2005 (O157:H7 and O26:H11-O80:H2).

- **Outside France**

Clustered cases in a family context are not usually the subject of publications. A review of published EHEC outbreaks occurring since 2006 highlighted the involvement of serogroups O157, O103, O26 and O145 in Europe, the USA and Japan (Table D). These outbreaks were linked, either epidemiologically or microbiologically, with conventional modes of EHEC transmission (person-to-person, foodborne and by direct contact with farm animals) (Table D).

The serotype and full profile of virulence genes were not published for all the EHEC strains isolated from these recent outbreaks, making it difficult to compare some of them with the major typical EHECs defined by AFSSA in 2008. Among the recent outbreaks described in publications, only one was attributed to a strain of serotype O103:H25 not appearing in the list of major typical EHECs defined by AFSSA in 2008 (Table D).

**Table D: New outbreaks of EHEC which have been described in the international literature since the publication of AFSSA's Opinion of 15 July 2008.**

| STEC serogroup/serotype | Country (Year) | Mode of transmission* | Number of cases | Reference |
|---|---|---|---|---|
| O157 | England (2009) | Contact with farm animals (petting zoo) | 36 | (Wise 2009) |
| O157:H- *stx1 stx2 eae* | Netherlands (2008-9) | Foodborne (raw beef) | 20 | (Greenland, de Jager *et al.* 2009) |
| O157:H- | United States (2007) | Contact with farm animals (petting zoo) | 7 | (Centre for Disease Prevention and Control 2009) |
| O157:H- *stx1 stx2 eae* | Netherlands - Iceland (2007) | Foodborne (lettuce) | 50 | (Friesema, Sigmundsdottir *et al.* 2008) |
| O157 *stx1 stx2* | England (2007) | Foodborne (chicken and herb sandwich) | 12 | (Whittaker, Sopwith *et al.* 2009) |
| O157:H7 *stx1 stx2* | Japan (2006) | Contact with farm animals (dairy farm) | 4 | (Muto, Matsumoto *et al.* 2008) |
| O157:H7 *stx2* | United States (2006) | Foodborne (spinach) | 205 | (Uhlich, Sinclair *et al.* 2008) |
| O103 *stx1* | Japan (2006) | Person-to-person (crèche) | 8 | (Muraoka, Okazaki *et al.* 2007) |
| O103:H25 *stx2* | Norway (2006) | Foodborne (lamb sausage) | 17 | (Schimmer, Nygard *et al.* 2008) |
| O26:H11 *stx1 stx2 eae* | Denmark (2007) | Foodborne (organic beef sausage) | 20 | (Ethelberg, Smith *et al.* 2009) |
| O26 *stx2 eae* / O145 *stx1 eae* | Belgium (2007) | Foodborne (pasteurised milk ice cream) | 12 | (De Schrijver, Buvens *et al.* 2008) |
| O26:H11 *stx1* | Japan (2006) | Person-to-person (crèche) | 6 | (Sonoda, Tagami *et al.* 2008) |

* Mode of transmission determined according to microbiological and epidemiological evidence, or epidemiological evidence alone.

### 4.3.3. Advances in knowledge of the molecular characterisation of EHECs

The list of virulence factors and mechanisms involved in the pathogenicity of EHEC strains is not yet fully known: although production of Stx toxin and colonisation of colonic mucosa are necessary, they are not sufficient to induce symptoms in humans. Many other potential virulence factors have been described. The combination(s) of virulence factors involved in the pathogenicity of EHEC strains remain to be determined and one of the major challenges of the molecular characterisation of isolates, especially in food, is how to distinguish between non-pathogenic strains and those which should be considered pathogenic, among the STECs. In the light of new knowledge available since the publication of AFSSA's Opinion of 15 July 2008, all the available data on virulence factors have been re-analysed.

- **Shiga toxins (Stx)**
  Shiga toxins are the primary virulence factors of EHECs. The Stx family contains all toxins with a similar structure (type $A_1B_5$ hexapeptides) and similar biological activity (AFSSA 2008). On the basis of their differing toxicity *in vitro* and *in vivo,* their amino acid sequence or nucleotide sequence of *stx* genes, two major types of Shiga toxin, Stx1 and Stx2, and many variants of Stx1 or Stx2, have been identified (AFSSA 2008).
  The variant type may reflect both the origin of the strains (sheep, pigs), their phylogeny, but also their pathogenicity (AFSSA 2008). *Stx* genes are carried by phages within the chromosome (or prophages) of the EHEC strains (Herold, Karch *et al.* 2004). Prophages encoding Stx toxins have been identified in *E. coli* but also in *S. dysenteriae* type 1, *Citrobacter freundii, Enterobacter cloacae* or *S. flexneri* (Herold, Karch *et al.* 2004). These phages can insert their DNA into the chromosome of the host bacterium at a single preferential site, which varies depending on the phage type (Schmidt 2001), although other secondary integration sites have also been described (Herold, Karch *et al.* 2004; Serra-Moreno, Jofre *et al.* 2007). Few integration sites for phages carrying the *stx* genes are known to date but many potential sites have been identified in the chromosome of *E. coli* (Hayashi, Makino *et al.* 2001). Several studies have shown that the *stx* genes could be transmitted within the *E. coli* population (Herold, Karch *et al.* 2004; Imamovic, Jofre *et al.* 2009; Muniesa and Jofre 2000). This phenomenon is also common in many EHEC strains belonging to various serotypes, as well as in other *Enterobacteriaceae* such as *Citrobacter* sp. (Schmidt, Montag *et al.* 1993) or *Enterobacte*r sp. (Paton and Paton 1996). The conversion of strains of *E. coli* has been demonstrated *in vitro* (Imamovic, Jofre *et al.* 2009; Muniesa and Jofre 2000; Watarai, Sato *et al.* 1998) as well as *in vivo* in the gastrointestinal tract of mice (Acheson, Reidl *et al.* 1998), ruminants (Cornick, Helgerson *et al.* 2006) and humans (Bielaszewska, Prager *et al.* 2007). Thus, the transfer of *stx* genes within the *E. coli* population could be a frequent phenomenon and could foster the emergence of new EHEC strains that are pathogenic for humans.

- **Intimin**
  Colonisation of the digestive tract is a major stage in the pathogenesis of EHECs. In typical EHECs, it is characterised by the development of attachment-effacement (A/E) lesions in enterocytes resulting from the combined action of proteins encoded by genes clustered within the LEE (McDaniel, Jarvis *et al.* 1995). The *eae* gene is one of the major genes conserved within the LEE (Bertin, Boukhors *et al.* 2004) and it encodes Intimin, a bacterial outer membrane protein that is involved in close adhesion of the bacterium to the enterocyte.
  Several variants of Intimin have been identified (AFSSA 2008). These different variants may be involved in cell tropism, host specificity and thus in the pathogenicity of EHECs (AFSSA 2008).
  Therefore, some variants (γ and ε Intimin) appear to be more specifically expressed by EHEC strains (Fitzhenry, Pickard *et al.* 2002; Oswald, Schmidt *et al.* 2000) but this association is not exclusive since these variants, like most other variants, are also found in EPEC strains and non-pathogenic strains (strains of animal origin not associated with symptoms in humans, or strains from other bacterial species) (Higgins, Frankel *et al.* 1999; Oswald, Schmidt *et al.* 2000).

Furthermore, certain variants appear to be associated with certain serotypes (AFSSA 2008; Beutin, Krause *et al.* 2004).
Nevertheless, this association is not exclusive: the same single serogroup may be associated with different variants of Intimin (Jores, Rumer *et al.* 2004). In addition, because variants of Intimin are not routinely screened for in the EHEC strains isolated from patients, as is the case in France, the number of EHEC strains studied is sometimes limited, especially for the least common serotypes and variants.

- **Other adhesion factors**

LEE-negative strains are generally non-pathogenic, but some of them have nevertheless been associated with cases of HUS (atypical EHECs). These atypical strains therefore have other adhesion factors which enable them to colonise the colonic mucosa as efficiently as the A/E.
Several potential adhesins have been described in atypical EHEC strains such as the adhesin Saa (*"STEC autoagglutinating adhesin")* from O113:H21 strains isolated in Australia in 1998 (Paton, Srimanote *et al.* 2001) or the plasmid adhesion factors of O111:H2 strains isolated in France in 1996 (Morabito, Karch *et al.* 1998),

Other potential adhesins have also been highlighted, both in atypical and typical EHEC strains:

- Fimbrial adhesins:
  * Lpf *fimbriae* (*"long polar fimbriae")* described in typical O157:H7 (Torres, 2002) and atypical O113:H21 strains (Doughty, Sloan *et al.* 2002);
  * Sfp *fimbriae* (*"sorbitol-fermenting EHEC O157 fimbriae")* demonstrated only in typical sorbitol-fermenting O157:HNM strains (Brunder, Khan *et al.* 2001; Friedrich, Nierhoff *et al.* 2004; Toma, Martinez Espinosa *et al.* 2004);
  * Proteins encoded by the genes of the genomic islands OI-1, OI-47, OI-141 and OI-154 (Shen, Mascarenhas *et al.* 2005) demonstrated in typical O157:H7 and O157:HNM strains;
  * Type IV *pilus* described in atypical O113:H21 and O48:H21 strains (Srimanote, Paton *et al.* 2002).

- Non-fimbrial adhesins:
  * Iha protein (*"IrgA homologous adhesin"),* identified in typical O157:H7 strains (Tarr, Bilge *et al.* 2000) as well as among atypical O113:H21 strains (Schmidt, Zhang *et al.* 2001);
  * OmpA adhesin (for *"outer membrane protein A")* described in typical O157:H7 strains (Torres and Kaper 2003);
  * Efal adhesin (for *"EHEC factor for adherence")* described in typical O111:H- (Nicholls, Grant *et al.* 2000) and O157:H7 strains (Morabito, Tozzoli *et al.* 2003);
  * ToxB protein demonstrated in typical strains (primarily O157 and O26) (Tatsuno, Horie *et al.* 2001; Tozzoli, Caprioli *et al.* 2005).

Thus, many proteins that may play a role in the colonisation of the digestive tract by EHECs have been described. However their involvement in the pathogenesis of strains remains to be demonstrated.

- **Other potential virulence factors**

Other potential virulence factors, encoded by genes found on the chromosome, on mobile genetic elements (plasmids, phages or pathogenicity islands) have been reported in EHEC strains (Croxen and Finlay 2010; Gyles 2007).

They include:

- toxins such as enterohaemolysin (Ehx), the enteroaggregative *E.coli* heat-stable toxin 1 EAST 1, subtilase cytotoxin (SubAB), the cyclomodulins CDT (for *"Cytolethal Distending Toxins")* and CIFs (for *"Cycle Inhibiting Factors"*);

- proteases such as the serine protease EspP, the catalase peroxidase KatP and the metalloprotease StcE;

- iron uptake systems, including the siderophore encoded by the pathogenicity island HPI (for *"High pathogenicity Island"*);

- mechanisms of resistance to gastric acidity;

- ureases;

- proteins of unknown function such as Nle effectors (for *"Non-LEE encoded effector"*).

The list of potential virulence factors continues to grow, but so far, their respective roles in the pathogenesis of EHECs have not been demonstrated.

- **Particular case of *"O Islands"***

  O Islands (OIs) are gene islands or segments found in the genome of EHECs (including *E. coli* O157:H7) but not in the genome of non-pathogenic strains of *E. coli* (AFSSA 2008). In total, 177 islands have been identified (Perna, Plunkett *et al.* 2001) some of which contain potential virulence factors, hence their classification as pathogenicity islands.

  Some islands include genes encoding type III effectors called Nle (Tobe, Beatson *et al.* 2006). The distribution of *nle* genes within the islands varies from one strain to another, and it can be noted that the more *nle* genes an island contains (i.e. the more complete it is), the more the symptoms associated with the strains containing them are serious. This observation is valid for three pathogenicity islands: OI-57 OI-71 and OI-122 (Coombes, Wickham *et al.* 2008; Karmali, Mascarenhas *et al.* 2003). A total of 14 *nle* genes (*nleA*, *nleB*, *nleC*, *nleE*, *nleF*, *nleG*, *nleG2-1*, *nleG2-3*, *nleG5-2*, *nleG6-2*, *nleG9*, *nleH1-2*, *nleH1-1* and *ent/espL2*) were identified as being more frequently found in EHEC strains than in STEC strains that had never caused an outbreak or case of HUS (Coombes, Wickham *et al.* 2008). Moreover, these genes contribute additively to virulence ("dose effect") since the strains causing either HUS or outbreaks contain a greater number of *nle* genes than non-virulent strains.

  Very recently, a genetic signature consisting of five genes (*eae*, *ent/espL2*, *nleB*, *nleE* and *nleH1-2*) was proposed for identifying EHEC strains (Bugarel, Beutin *et al.* 2010). However, some caution should be exercised because the presence of these five genes has also been reported in STEC strains belonging to the seropathotypes D and E, considered as less pathogenic or presumed to be non-pathogenic for humans (Coombes, Wickham *et al.* 2008).

Shiga toxins and Intimin are the two main proteins involved in pathogenicity of EHECs, although not all variants of Stx or Intimin appear to have the same toxicity or the same specificity for humans.

However, several other as yet unknown or poorly determined factors appear to be involved in the pathogenicity of EHECs. Furthermore, although the characteristics of the strain in question, in particular all the virulence factors expressed, are probably critical, the infection process of EHECs is multifactorial and also depends on host-related factors.

For a STEC strain isolated outside a clinical setting in humans, the consensus today is that it can be considered pathogenic if it has at least one *stx* gene *(stx1* and/or *stx2)* and the *eae* gene.

## 5. CONCLUSION

The French Food Safety Agency has concluded that the analysis of epidemiological data shows that some serotypes of enterohaemorrhagic *E. coli* isolated from humans are more frequently associated with a serious illness. In most cases, these serotypes have specific molecular characteristics, described in AFSSA's Opinion of 15 July 2008 as those of major typical EHECs.

The 2008 definition of major typical EHECs therefore remains valid, although other serotypes or sometimes atypical EHECs may be isolated more rarely in human infections.

However, AFSSA would like to emphasise that during the bacteriological examination of food, conducted outside a clinical context in humans, it is in fact the detection of the different virulence factors or markers within the same strain that enable its pathogenicity to be estimated. Therefore, the strain should be considered as**:**

- highly pathogenic when it has the characteristics of a major typical EHEC (presence of the virulence genes *stx1* and/or *stx2* and *eae,* and belonging to one of the following serotypes and their non-motile derivatives: O157:H7, O26:H11, O145:H28, O103:H2 and O111:H8),

- pathogenic when it has the characteristics of a typical EHEC (presence of the virulence genes *stx1* and/or *stx2* and *eae*).

**The Director General**

**Marc MORTUREUX**

## KEY WORDS

Enterohaemorrhagic *Escherichia coli* (EHEC), enteropathogenic *Escherichia coli* (EPEC), gastrointestinal infection, haemolytic uremic syndrome (HUS), outbreak, Shiga toxin-producing *Escherichia coli* (STEC), foods

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Annex - Table of EHEC serogroups/serotypes, identified in cases of HUS and thrombotic micro-angiopathy in adults by the NCR for *E. coli* and *Shigella* and its affiliated laboratory between 2007 and 2009.

| Serogroups/Serotypes* | Total | 2007-2009 | |
|---|---|---|---|
| | | eae+ (typical) | eae- (atypical) |
| O157 | 59 | 57 | 2 |
| O26 (including one O26:H11) | 19 | 19 | 0 |
| O145 (including one O145:H2) | 2 | 2 | 0 |
| O103:H2 | 1 | 1 | 0 |
| O111 (including one O111:H8 *eae+*) | 2 | 1 | 1 |
| O91:H10 | 1 | 0 | 1 |
| O5:H18 | 1 | 0 | 1 |
| O7:R6 | 1 | 0 | 1 |
| O55 | 1 | 1 | 0 |
| O76:H19 | 1 | 0 | 1 |
| O80:H2 | 1 | 1 | 0 |
| O86:F27 | 1 | 1 | 0 |
| O114 | 1 | 1 | 0 |
| O121:H2 | 1 | 1 | 0 |
| O121:H19 | 8 | 0 | 8 |
| O123:H2 | 1 | 1 | 0 |
| O126 | 1 | 1 | 0 |
| O174:H2 | 1 | 0 | 1 |
| O174:H21 | 4 | 0 | 4 |
| NST** | 27 | 19 | 8 |
| *Total* | 134 | 106 | 28 |

* The serogroup (or serotype) was obtained by agglutination with the serum panel used by the NCR for *E. coli* and *Shigella* and its affiliated laboratory or determined by molecular strain typing at the NCR.

** NST: Non-serotypeable.