SCIENTIFIC REPORT OF EFSA

Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Europe: Taking Stock[1]

European Food Safety Authority[2, 3]

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ABSTRACT

On 21 May 2011, Germany reported an ongoing outbreak of Shiga-toxin producing *Escherichia coli* (STEC), serotype O104:H4. From an initial case control study, the outbreak was associated with the consumption of fresh salad vegetables. Subsequent investigations showed that the risk of infection was significantly associated with the consumption of fresh sprouted seeds rather than with other fresh vegetables. A tracing back and tracing forward study showed that all of the clusters for which there was sufficient information, could be attributed to consumption of sprouted seeds from a single sprouted seed producer in Germany. Investigation of the production site showed no evidence of environmental contamination. Employees were found to be infected, but since they had not become ill prior to the outbreak, it was concluded that they were not the source of the food contamination. Hence, contaminated seeds used for the sprout production were the most likely source. Several varieties of seeds were used, and sprouts thereof were sold as a mixture. Subsequently, a cluster of patients with bloody diarrhoea was reported, after having participated in a local event in France on 8 June. Consumption of sprouted seeds was also associated with occurrence of the disease in this cluster. Furthermore, the STEC isolates responsible for the outbreaks in France and Germany were found to be indistinguishable. It was therefore concluded that there was a common source for both outbreaks. A comparison of the back tracing information on the seeds from the French and German outbreaks led to the conclusion that a specific consignment (lot) of fenugreek seeds imported from Egypt was the most likely link between the outbreaks, although it could not be excluded that other lots from the same exporter and importer were also implicated. STEC O104 is a very rare serogroup in humans in the EU and worldwide. Sporadic cases in the EU have been previously linked to travel to North Africa, the Middle East and Central Asia. On 26 July, the Robert Koch Institute declared the outbreak finished. A total of 3911 cases have been reported to the ECDC and WHO, linked to the outbreaks, to date.

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KEY WORDS

E. coli, outbreak, sprouted seeds, fenugreek, STEC, VTEC, EHEC, EAggEC, ESBL

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SUMMARY

On 21 May 2011, Germany reported an ongoing outbreak of Shiga-toxin producing *Escherichia coli* (STEC), serotype O104:H4. As of 27 July, 3126 cases of diarrhoeal disease caused by STEC *E. coli* O104:H4 (probable and confirmed), including 17 deaths linked to the outbreak in Germany and occurring in the EU (including Norway) have been reported to the European Centre for Disease and Control (ECDC). In addition, in the EU 773 cases of haemolytic uraemic syndrome (HUS) caused by this bacterium were also reported, including 29 deaths, and linked to the German outbreak. At this time of reporting, a further 119 suspect cases including 4 deaths are also associated with this outbreak. In addition, outside the EU 8 cases of STEC and 5 cases of HUS, including 1 death have been reported in the USA, Canada and Switzerland through the international health regulations (IHR), all with recent travel history to Germany. Based on the most recently reported information (last updated on 26 July 2011), the clinical onset of the last outbreak-related case in Germany was 4 July 2011.

Shortly after the onset of the outbreak in Germany, case-control studies conducted by the Robert Koch Institute (RKI) demonstrated that clinical disease was statistically significantly associated with the consumption of fresh salad vegetables. The high proportion of adult women among cases, was consistent with fresh salad vegetables as the source of infection. Later, a detailed cohort study demonstrated an association with sprouted seeds.

A tracing back and tracing forward study showed that most of the clusters, and all of the clusters for which there was sufficiently detailed food consumption data, could be attributed to consumption of sprouted seeds from one producer in Germany. Investigation of the production site showed no evidence of environmental contamination. Some employees were found to be infected, but reported not to have become ill prior to the outbreak and hence it was concluded that they were not the source of the contamination. This left the seeds used for the sprout production as the prime suspect vehicle of infection; however it was not possible to identify a single seed source since different species were used to produce the sprouted seeds, which were sold as several different mixtures.

On the 24 June, France reported to the Rapid Alert System for Food and Feed (RASFF[4]), a cluster of patients with bloody diarrhoea, after having participated in an event in the Commune of Bègles near Bordeaux on the 8 June. At the time of issue of this report, there were 2 confirmed STEC cases and 9 cases with HUS reported to ECDC, with 4 further cases of non-HUS suspected. Eleven of these patients, 7 women and 4 men, between 31 and 64 years of age, had attended the same event in Bègles. Infection with *E. coli* O104:H4 was confirmed for 12 of the 15 cases. Epidemiology studies on the French outbreak also implicated sprouted seeds as the outbreak vehicle.

The phenotypic and genotypic characterisation of the *E. coli* O104:H4 indicated that the isolates from the French and German outbreaks were common to both incidents. Hence, it was concluded that the same strain was involved in the outbreaks both in Germany and in France, strongly indicating a common source.

A tracing back initiated to find the common food source for both outbreaks revealed fenugreek seeds to be common to the 2 outbreaks. The comparison of the back tracing information on the seeds from the French and German outbreaks led to the conclusion that a specific consignment (lot) of fenugreek seeds imported from Egypt was the most likely link between the outbreaks, although it could not be excluded that other lots imported by the same importer/exporter might be implicated.

Data concerning the trace back and trace forward were exchanged through the RASFF, allowing the Member States and European institutions to receive up to date information.

The actual cause or route of contamination of the seed has not been demonstrated. However, based on epidemiological and microbiological investigations within the EU as well as from previous sprouting

[4] RASFF: http://ec.europa.eu/food/food/rapidalert/index_en.htm

seed-related outbreaks, it is likely that contamination occurred during seed production. This part of the investigation would need to extend beyond the point of EU import to include the site(s) of production.

The published data for STEC O104 are scarce as this is a very rare serogroup infecting humans in Europe and globally. According to the information reported to ECDC, there were 10 reported cases of STEC O104 infection in the EU Member States and Norway during 2004-2010 from: Austria (1 case in 2010), Belgium (1 case in 2008), Denmark (1 case in 2008), Finland (1 case in 2010), France (1 case in 2004), Norway (1 case in 2006, 3 cases in 2009), and Sweden (1 case in 2010). Moreover, a paediatric case of HUS which occurred in Italy in 2009 has now been associated with STEC O104, so giving a total of 11 cases.

Five of the 10 cases between 2004 and 2010 were related to travel outside the EU; the countries of origin of the infection being Afghanistan (2008), Egypt (2010), Tunisia (2009, 2010) and Turkey (2009). Only 3 of the STEC O104 strains isolated from these cases were of serotype O104:H4 (in Finland in 2010, in Italy in 2009, and in France in 2004). As for the outbreak strain, the STEC O104:H4 strains isolated in Italy and in Finland were both positive for genetic markers of enteroaggregative adhesion, but differed from the 2011 epidemic strain in that they were negative for extended-spectrum beta-lactamase production. The Finnish case was travel-related, with infection acquired in Egypt; whereas the Italian case had a recent history of travel to Tunisia. The origin of the source of infection for the French case was not reported.

In addition to those cases reported to ECDC, a review of the scientific literature revealed that STEC O104:H4 has been isolated twice in Germany in 2001 and once in Korea in 2005. The German isolates differed from the 2011 outbreak strain.

E. coli O104:H4 was not isolated from any batches of the suspect fenugreek seeds. The inability to demonstrate the presence of *E. coli* O104:H4 in the suspect seeds is not unexpected. It is possible that contaminated seeds were no longer in stock when sampling took place, or even if present were contaminated at a level which made isolation of the organism impossible. However, this does not mean that enterobacteriaceae would not have been present in seeds and sprouted seeds. Previous studies have shown enterobacteriaceae to be present on the surface of the tissue of the plants and that they can also be internalised within the plant (*e.g.* at primary production, through irrigating with contaminated water or application of organic fertilizer not properly treated and still containing enteric pathogens). In this regard, it is important to underline that a negative laboratory test does not prove the absence of a pathogen, which may be unevenly distributed within the food matrix, perhaps at low levels. This is particularly true when dealing with seeds: the matrix is made up of particles (seeds) which individually may become contaminated and dispersed in large lots. Similarly, favourable physico-chemical conditions to support survival or growth may not be homogeneously distributed.

The preparation of fresh sprouted seeds seldom includes a step where bacterial contamination is eliminated. Hence, food preparation of fresh sprouted seeds is based on the understanding that they are sold as ready-to-eat, *i.e.* safe to eat as is, or following only minimal preparation. For fresh produce, this assumes and relies on a production process which prevents contamination and an ability to detect contamination when it occurs. These conditions have proven not to be satisfied in this case. The fact that the sampling and bacteriological methods might fail to detect the presence of pathogens such as STEC O104:H4 and *Salmonella* spp. emphasises the importance of good production and handling practices, as the public health protection associated with such a criterion is questionable given the difficulty of tracing pathogens in seeds.

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BACKGROUND AS PROVIDED BY EFSA

On the 21 of May 2011, Germany reported an ongoing outbreak of Shiga-toxin producing *Escherichia coli*- bacteria (STEC[5][6]), serotype O104:H4. As of 27 July, 3126 cases of diarrhoeal disease caused by STEC *E. coli* O104:H4 (probable and confirmed), including 17 deaths linked to the outbreak in Germany and occurring in the EU (including Norway) have been reported to the ECDC. In addition, 773 cases of haemolytic uraemic syndrome (HUS) caused by this bacterium were also reported, including 29 deaths, and linked to the German outbreak. At this time of reporting, a further 119 suspect cases including 4 deaths are also associated with this outbreak[7].

On the 24 of June, France reported[8] a cluster of patients with bloody diarrhoea, after having participated in an event in the Commune of Bègles near Bordeaux on the 8 of June. At the time of issue of this report, there were 2 confirmed STEC cases and 9 cases with HUS reported to ECDC, with a further 4 suspected cases of non-HUS. Eleven of these patients had attended the same event in Bègles. Infection with *E. coli* O104:H4 was confirmed for 12 of the 15 cases. Epidemiology studies on the French outbreak also implicated sprouted seeds as the outbreak vehicle.

Both events have resulted in numerous investigations to establish the extent, vehicle, source, and cause of this outbreak. These investigations have led to the conclusion that fenugreek seeds imported into Europe from Egypt were the most likely source of contamination, and sprouted seeds the most likely vehicle. The objective of this review is to provide an overview of key information that was gathered and to reference where this information is presented in detail.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The Emerging Risks unit (EMRISK) is requested to coordinate the following activities in collaboration with the Scientific Support and Assessment Unit, Biological Hazards Unit, Biological Monitoring Unit and Communications;

1) In collaboration with the French and German authorities, and the EU Reference Laboratory on *E. coli*, prepare a Scientific Report summarising the strain characteristics, epidemiological investigations, and analytical methods for foods linked to this outbreak. Make extensive reference to the reports produced by the national authorities and European institutions concerning this outbreak.

2) In collaboration with the units involved in EFSA's outbreak response, produce a discussion paper summarising EFSA's contribution to resolving the outbreak, and highlighting lessons learnt for EFSA, for collaboration at MS level and at the level of EU coordination. This paper will address both EFSA's scientific work and risk communications activities undertaken in coordination with ECDC, Member States and the European Commission.

This report only addresses the first of the terms of reference (ToR). The second ToR will be addressed in a separate document.

[5] European Food Safety Authority; Urgent advice on the public health risk of Shiga-toxin producing *Escherichia coli* in fresh vegetables. EFSA Journal 2011; 9(6):2274. [50 pp.]

[6] In the EU and as reflected in EFSA's work on zoonoses, Shiga-toxin producing *Escherichia coli* are referred to as VTEC (verotoxin-producing *E. coli*), but the term STEC is used for this outbreak as it is in line with terminology used by WHO and other organisations.

[7] http://www.ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f-77d4-4ad8-b6d6-bf0f23083f30&ID=1166&RootFolder=/en/activities/sciadvice/Lists/ECDC Reviews&MasterPage=1

[8] RASFF Alert Notification 2011.0842[8] RASFF Alert Notification 2011.0842, Available at https://webgate.ec.europa.eu/rasff-window/portal/index.cfm?event=notificationsList

ASSESSMENT

1. Introduction

On the 21 of May 2011, Germany reported an ongoing outbreak of Shiga-toxin producing *Escherichia coli*- bacteria (STEC[9][10]), serotype O104:H4. As of 27 July, 3126 cases of diarrhoeal disease caused by STEC *E. coli* O104:H4 (probable and confirmed), including 17 deaths linked to the outbreak in Germany and occurring in the EU (including Norway) have been reported to the European Centre for Disease Prevention and Control (ECDC). In addition, 773 cases of haemolytic uraemic syndrome (HUS) caused by this bacterium were also reported, including 29 deaths, and linked to the German outbreak. At this time of reporting, a further 119 suspect cases including 4 deaths are also associated with this outbreak [11]. Further details on the German cases are available from the Robert Koch Institute[12] (RKI, 2011).

On the 24 of June, France reported[13] a cluster of patients with bloody diarrhoea, after having participated in an event in the Commune of Bègles near Bordeaux on the 8 of June. At the time of issue of this report, there were 2 confirmed STEC cases and 9 cases with HUS reported to ECDC, with a further 4 suspected cases of non-HUS. Eleven of these patients, 7 women and 4 men, between 31 and 64 years of age, had attended the same event in Bègles. Infection with *E. coli* O104:H4 was confirmed for 12 of the 15 cases. Epidemiological studies on the French outbreak also implicated sprouted seeds as the outbreak vehicle.

Both events have resulted in investigations to find the source of this outbreak which resulted in the conclusion that contaminated fenugreek seeds imported into Europe from Egypt were the most likely source. The objective of this review is to provide an overview of key information that was gathered and to reference where this information is presented in detail. The paper covers what is known about:

- the hazard, in this case the bacteria causing the illness;
- the vehicle of infection, *i.e.* what people ate that made them ill;
- the source, *i.e.* where the bacterium came from ;
- the cause, *i.e.* how sufficient contamination to cause the outbreak got from the source to the vehicle.

[9] European Food Safety Authority; Urgent advice on the public health risk of Shiga-toxin producing *Escherichia coli* in fresh vegetables. EFSA Journal 2011; 9(6):2274. [50 pp.]

[10] In the EU and as reflected in EFSA's work on zoonoses, Shiga-toxin producing *Escherichia coli* are referred to as VTEC (verotoxin-producing *E. coli*) but the term STEC is used for this outbreak as it is in line with terminology used by WHO and other organisations.

[11] http://www.ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f-77d4-4ad8-b6d6-bf0f23083f30&ID=1166&RootFolder=/en/activities/sciadvice/Lists/ECDC Reviews&MasterPage=1

[12] http://www.rki.de/cln_169/nn_217400/EN/Home/PM__EHEC.html

[13] RASFF Alert Notification 2011.0842[13] RASFF Alert Notification 2011.0842, Available at https://webgate.ec.europa.eu/rasff-window/portal/index.cfm?event=notificationsList

2. Characterisation of the *E. coli* O104:H4 strain isolated in the current outbreak

For a general recent brief review of characteristics of *E. coli* O104:H4 that are particularly relevant to this outbreak, see EFSA (2011a).

Germany

The outbreak strain is a Shiga toxin producing *Escherichia coli* (STEC) that belongs to serotype O104:H4. Detailed information on the outbreak strain characteristics can be found at the Robert Koch-Institut (RKI) website[14]. Briefly, its microbiological characteristics are as follows:

- Shigatoxin 1 (*stx1*) not present
- Shigatoxin 2 (*stx*2a) present
- Intimin (*eae* gene) not present
- Enterohemolysin (*hlyA*) not present
- EAggEC (enteroaggregative *E. coli*) virulence plasmid:
  - *aat*A present (ABC-transporter protein gene);
  - *agg*R present (master regulator gene of virulence-plasmid genes);
  - *aap* present (secreted protein dispersin gene);
  - *aggA* present (AAF/I-fimbral subunit-gene);
  - *agg*C present (AAF/I-fimbral operon-gene);
- Multi-locus sequence typing (MLST) Sequence Type: ST678 (adk 6, fumC6, gyrB 5, icd 136, mdh 9, purA 7, recA 7);
- Antimicrobial resistance profile: resistant to ampicillin, amoxicillin/clavulanic acid, piperacillin/sulbactam, piperacillin/tazobactam, cefuroxime, cefuroxime-axetil, cefoxitin, cefotaxime, cetfazidime, cefpodoxime, streptomycin, nalidixic acid, tetracyclin, trimethoprim/sulfamethoxazol;
- The strain carries plasmid-borne $bla_{\text{CTX-M-15}}$ and a $bla_{\text{TEM-1}}$ genes;
- The macrorestriction-pulsed field gel electrophoresis (PFGE)-pattern (XbaI) of the current STEC O104:H4 is different compared to HUSEC 041 (Robert Koch Institute, Personal Communication);

In conclusion, the STEC O104:H4 outbreak strain shows an unusual combination of virulence factors of STEC and EAggEC which has only been reported for a group of strains of serotype O111:H2 (Morabito et al., 1998) isolated in France during an outbreak of HUS (Boudailliez et al., 1997). As in the present outbreak, the association of the O111 strains with severe disease supports the view that this unusual combination of virulence factors might confer a very high degree of virulence. It is also noteworthy that the outbreak described by Boudailliez et al. (1997), was ascribed to a person-to-person transmission, without any evidence of foodborne transmission.

Whole genome sequencing data confirms that the German outbreak strain is an EAggEC which can be distinguished from those of other O104:H4 strains because it contains a prophage encoding Shiga toxin 2 and a distinct set of additional virulence and antibiotic-resistance factors which have been acquired by horizontal genetic exchange (Rasko et al., 2011).

14 http://www.rki.de/cln_160/nn_217400/EN/Home/EHECO104,templateId=raw,property=publicationFile.pdf/EHECO104.pdf

France

The strain of *E. coli* O104:H4, isolated from 5 HUS cases, all of whom consumed sprouted seeds served at an event in Bègles (near Bordeaux, South-west France) on 8 June 2011, exhibits the following characteristics (ANSES, 2011):

- The strain contains the *stx2 and aggR* genes, but not the genes coding for intimin (*eae*), haemolysin A (*hlyA*) and EAST1 toxin (*astA*);
- The antimicrobial susceptibility pattern of the strain is as follows: resistant to ampicillin, cefotaxime, ceftazidime, cotrimoxazole, streptomycin, trimethoprim, sulfamethoxazole, tetracyclin, and nalidixic acid. Sensitive to chloramphenicol, ciprofloxacin, gentamicin, imipenem, and kanamycin;
- The strain contains extended-spectrum beta-lactamase (ESBL) $bla_{\text{CTX-M-15}}$ (group 1) gene and the penicillinase bla_{TEM} gene.

Thus, information on the microbiological characterisation of the isolates implicated in the French outbreak indicate that many characteristics (*stx*2 positive, *eae* negative, *hly*A negative, multi-resistance pattern to antimicrobials) are common with the German outbreak strain. In addition, the 2 molecular techniques (Repetitive sequence based Polymerase Chain Reaction (Rep-PCR) and pulsed-field gel electrophoresis (PFGE)) used to compare the outbreak strain in France and isolates of *E. coli* O104:H4 from 2 imported cases in France linked to the *E. coli* O104:H4 outbreak in Germany in May and June 2011, showed that the isolates were indistinguishable by these methods (Gault et al., 2011).

3. Previous worldwide occurrence of *E. coli* O104:H4

ECDC and EFSA reported on the previous occurrence of STEC O104:H4 (ECDC, EFSA 2011). The text below is extracted from this document.

The published data for STEC O104 is scarce as this is a very rare serogroup in humans in Europe and globally. According to the information reported to ECDC, there were 10 reported cases infected with STEC O104 in the EU Member States and Norway in the period 2004-2009. These STEC O104 cases have been reported from Austria (1 case in 2010), Belgium (1 case in 2008), Denmark (1 case in 2008), Finland (1 case in 2010), France (1 case in 2004), Norway (1 case in 2006, 3 cases in 2009), and Sweden (1 case in 2010) (ECDC and EFSA, 2011). Moreover, a paediatric case of HUS which occurred in Italy in 2009 has now been associated with STEC O104 (Scavia et al., 2011), so giving a total of 11 cases.

Based on the known data, 56% of the cases were male and the age ranged from <1 year to 76 years. Two of the cases (18%) developed HUS. Five of the cases were travel related; the countries of origin of the infection being Afghanistan (2008), Egypt (2010), Tunisia (2009 and 2010) and Turkey (2009). Only 3 of the STEC O104 strains isolated from these cases were of serotype O104:H4 (in Finland in 2010, in Italy in 2009, and in France in 2004). Similar to the outbreak strain, the STEC O104:H4 strains isolated in Italy and in Finland were both positive for genetic markers of enteroaggregative adhesion, but negative for ESBL (extended-spectrum beta-lactamase) production. As mentioned, the Finnish case was travel-related, with the infection acquired in Egypt; whereas the Italian case had a recent history of travel to Tunisia (Scavia et al., 2011). The origin of the source of infection for the French case was not reported.

From a review of the scientific literature, STEC O104:H4 has been isolated twice in Germany in 2001 (Mellmann et al, 2008) and once in Korea in 2005 (Bae et al, 2006). The case in Korea was a 29-year old woman who developed HUS. The source of the infection was unknown. The STEC O104:H4 from Germany, with a MLST ST678, was isolated from an HUS case (Mellmann et al., 2008). Like

the current outbreak strain, the isolate was *stx*2-positive, *eae*-negative, and positive for enteroaggregative adhesion and EAggEC genetic markers. However, it was negative for ESBL production[15], and some other differences with the outbreak strain were reported (Bielaszewska et al., 2011).

4. Background on the outbreaks in the EU

This section complements the ECDC/EFSA rapid risk assessment[16] published on 29 June, 2011, and the ECDC rapid risk assessment update published on 8 July[17].

As of 27 July, 3126 cases of STEC caused by *E. coli* O104:H4 (probable and confirmed), including 17 deaths in the EU (including Norway), linked to the outbreak in Germany, have been reported to the ECDC. In addition, in the EU 773 cases of HUS have been reported, including 29 deaths, also linked to the German outbreak[18]. A further 119 cases of STEC and 4 deaths are suspected at this time of reporting. Switzerland has so far reported 5 non-HUS STEC cases through the international health regulations (IHR), the United States reported 5 HUS cases including 1 death and 2 non-HUS cases, all with recent travel history to Germany. Canada has also reported 1 non-HUS case with travel history to Germany.

As based on the last reported information (26 July 2011), the clinical onset of the last outbreak-related case in Germany was the 4 July 2011[19].

On the 24 June, France reported to the RASFF[20] a cluster of patients with bloody diarrhoea, after having participated in an event in the Commune of Bègles near Bordeaux on the 8 June. At the time of issue of this report, there were 2 confirmed STEC cases and 9 cases with HUS reported to ECDC, with 4 further cases of non-HUS suspected. Eleven of these patients, 7 women and 4 men, between 31 and 64 years of age, had attended the same event in Bègles. Infection with *E. coli* O104:H4 was confirmed for 12 of the 15 cases. Epidemiology studies on the French outbreak also implicated sprouted seeds as the outbreak vehicle.

An important development since 29 June comes from the results of the screening of children and staff in a school in Germany where 3 cases of HUS and a further 4 cases of STEC related diarrhoea had been identified. As most recently reported (5 August 2011)[21], the epidemic strain of *E. coli* was isolated from 22 of the 30 children tested, indicating a high level of occurrence of asymptomatic carriage. Three teachers at the school were found to be asymptomatic carriers of STEC as were 2 employees of the catering company supplying the school.

5. Epidemiological investigations into the vehicle, source and cause of the outbreak

Early after the onset of the outbreak in Germany, case-control studies conducted by the RKI demonstrated that the onset of clinical disease was linked to the consumption of fresh salad vegetables. Circumstantial evidence, such as the high proportion of adult women among the ill, was also

[15] http://www.ehec.org/index.php?hid=43&lang=de&pid=HUSEC
[16] http://ecdc.europa.eu/en/publications/Publications/2011June29_RA_JOINT_EFSA_STEC_France.pdf
[17] http://www.ecdc.europa.eu/en/publications/Publications/110712_TER_Risk_Assessement_Ecoli.pdf
[18] http://www.ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f-77d4-4ad8-b6d6-bf0f23083f30&ID=1166&RootFolder=/en/activities/sciadvice/Lists/ECDC Reviews&MasterPage=1
[19] http://www.rki.de/cln_110/nn_205760/DE/Content/InfAZ/E/EHEC/Info-HUS,templateId=raw,property=publicationFile.pdf/Info-HUS.pdf
[20] RASFF: http://ec.europa.eu/food/food/rapidalert/index_en.htm
[21] http://www.kreis-paderborn.org/kreis_paderborn/presse/2011/entries/erster-todesfall-ehec.php

consistent with vegetables being the vehicle of infection. Later, a detailed cohort study demonstrated an association with sprouted seeds[22].

In an attempt to identify the food source through microbiological examination, the responsible competent authorities of the German Federal States performed risk-based inspections of clusters (where a common meal or place of eating was identified) and took samples from foods and the environment at clusters, suppliers, whole sale markets, and at retail[23]. So far (26 July, 2011), none of the food samples taken during this monitoring activity, for which cross-contamination (from infected persons in the household) could be excluded, has been found positive for the STEC serotype O104:H4 (BfR, 2011b).

Consequently, it was decided that a co-ordinated initiative to identify the food source and stop the outbreak was needed. For this purpose, the German Task Force EHEC (Enterohaemorrhagic *Escherichia coli*) was established by the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV). Members of the Task Force EHEC were experts from the Federal States in Germany, from the Federal Office of Consumer Protection and Food Safety (BVL), the Federal Institute for Risk Assessment (BfR), the RKI and EFSA scientists. The Task Force EHEC was hosted in the crisis centre at the BVL (Task Force EHEC, 2011).

In a first phase, 2 different strategies were followed to identify the STEC contaminated food. The first approach examined 5 clusters in Germany (associated with hotels, restaurants or canteens), which were prioritized due to the availability of precise information on the food intake of the affected cases (*e.g.* tour parties, family celebrations etc.). A detailed trace back analysis was conducted for all salad ingredients (including sauces and dressings etc.) and toppings that had been served to the customers. The second approach was a trace forward analysis of the supply chains of a horticultural farm in Lower Saxony (Establishment A) in order to find out if any of the clusters were linked to sprouting seeds originating from there. The production site was suspected early on by the local authorities as a possible source of infection for several clusters. Furthermore, STEC O104:H4 was isolated from the faeces of employees of Establishment A, including 2 asymptomatic cases.

A total of 41 clusters were identified for which there was sufficient information on food intake in order to carry out the tracing. The analysis of the sprouted seed supply chains showed that for all 41 clusters, there was a common link to sprouted seeds from Establishment A (the trace forward approach). The results of the detailed trace back analysis at 5 clusters confirmed the findings of the trace forward analysis. It was shown that sprouted seeds from Establishment A had been delivered to all 5 clusters. Thus, the epidemiological investigations provided strong evidence that sprouted seeds from Establishment A were the vehicle of infection of the German STEC O104:H4 outbreak[24].

Further epidemiological investigations revealed that the disease occurrence coincided with consumption of either of 2 different sprouted seed mixtures, the “Keimspross-Mischung / Milde Mischung” (germ sprout or mild blend) or the “Würz-Mischung” (spicy blend). The mild blend contained 4 and the spicy 3 different kinds of sprouted seeds. Only lentil and fenugreek sprouts were present in both mixtures. Whereas lentil sprouts were also used in other sprouted seed mixtures, fenugreek sprouts were used for “Keimspross-Mischung / Milde Mischung” and “Würz- Mischung” only[25]. Hence, fenugreek sprouts were almost exclusively used for the 2 mixtures and only small amounts were sold unmixed.

The sprouting procedure of fenugreek seeds was examined in detail. Fenugreek seed sprouting was initiated twice per week, every week. The sprouting process took 3.5 days. After sprouting, the

[22] http://www.rki.de/cln_117/nn_217400/EN/Home/EHEC__Report,templateId=raw,property=publicationFile.pdf/EHEC_Report.pdf

[23] www.bvl.bund.de/taskforce_en

[24] www.bvl.bund.de/taskforce_en

[25] www.bvl.bund.de/taskforce_en

fenugreek seeds were mixed with other sprouted seeds to generate the "Keimspross-Mischung / Milde Mischung" and the "Würz-Mischung". It was assumed that fresh sprouted seeds were eaten after a maximum of 14 days following their production. Starting with the known dates of exposure of the cases from the selected 5 clusters and the delivery dates for the sprouted seeds to the place of exposure (hotels, restaurants or canteens) the possible production dates at Establishment A were assessed. A comparison with the total epidemiological curve of the outbreak confirmed the isolated production cycles as consistent with the whole outbreak. Establishment A used lot nos. 48088 and 8266 of fenugreek seeds for their production during April to mid-May 2011. The earlier one (lot no. 48088) was totally used up when sampling by the local authorities took place at Establishment A (Task Force EHEC 2011). Both lots were traced back to a single importer in Germany, who imported the seeds from Egypt.

Three of the personnel of Establishment A developed diarrhoeal symptoms consistent with an STEC infection, with earliest onset of disease on 6 May 2011. Two of the clinically ill staff worked part-time, but included days when fenugreek seeds and sprouts were handled. The outbreak strain STEC O104:H4, however, could only be detected in 1 of the 3 staff members. Moreover, within the course of the investigation of the environment of Establishment A, 2 more staff members were identified shedding the outbreak strain, both without any diarrhoeal symptoms. It was therefore questioned whether the personnel could be the origin of the infection. While the onset of disease of the first staff member who became ill occurred early in the outbreak, it occurred within days of the first occurrences of illness in other people having eaten sprouted seeds originating from Establishment A. Personnel from Establishment A were given a weekly package of sprouted seeds for private use, however, this may explain the early exposure of personnel from Establishment A, but does not support that they introduced the contamination into the production chain (BfR, 2011a). This does not exclude that they may have amplified the spread of the infection in Establishment A, or that an unidentified carrier or infected individual was responsible.

It should be noted that the tracing back investigations in Germany were done for all the types of seeds used in "Keimspross-Mischung / Milde Mischung" and "Würz-Mischung". Additionally, an inventory of all sprouted seed producers in Germany was established to gather information on the simultaneous use of the suspected seeds. Hence, intensive sampling and microbiological analysis of seeds and sprouts were performed. In the case of the occurrence of additional clusters, this information could then be used to refine the identification of the source of infection, as well as, if seed contamination were detected, a more rapid recall of all material.

The occurrence of the outbreak in France, without any direct link to the outbreak in Germany, but caused by the same STEC strain as in the German outbreak (Gault et al., 2011) and the fact that sprouting seeds were also strongly suspected as the source of the French outbreak (ANSES, 2011), considerably strengthened the hypothesis that, among possible sources, seeds were the most likely vehicle of the contamination. A cohort study of those attending the event was initiated to identify which specific foods were significantly associated with the onset of clinical disease in the French cluster and preliminary results revealed a significant association between consumption of sprouted seeds (fenugreek, rocket and white mustard) and infection. In addition, the study showed that none of the food handlers at the event or people who served the dishes had recently travelled to Germany, and none of the guests had recent contact with someone coming back from Germany[26].

Following this outbreak in France, EFSA was asked by the European Commission to support the Member States and coordinate activities to investigate the source of the outbreaks in France and Germany in order to allow risk managers to take the necessary and appropriate risk mitigating measures towards prevention of further outbreaks. For this purpose EFSA set up a task force composed of experts from the European Commission, affected EU Member States, the ECDC, WHO and FAO, as well as EFSA staff members. Data concerning the trace back and trace forward were

[26] http://www.invs.sante.fr/behweb/2011/03/pdf/n3.pdf

exchanged through the RASFF[27], allowing the MS and European institutions to receive up to date information. The study established one common link between all 41 of the identified German clusters and the French cluster through the import of fenugreek from Egypt by a single importer in Germany. The Importer had bought several large lots of these seeds over the last 48 months and distributed more than one lot at the same time.

The technical report published by this ESFA task force on 5 July (EFSA, 2011b) concluded that a specific lot (no. 48088) of fenugreek seeds imported from Egypt was the most likely common link; however the implication of other lots cannot be excluded. The exact point of contamination in the food chain has not been established, but took place at some point prior to leaving the Importer. The contamination of seeds with the STEC O104:H4 strain reflects a production or distribution process which allowed for contamination with faecal material of human and/or animal origin. Where exactly this took place has not been established. Typically such contamination occurs during production at the farm level, due to the use of natural fertiliser (e.g. animal manure or slurry) or contaminated irrigation water. In general, such types of contamination lead to bigger (e.g. transnational) outbreaks, while local contamination during storage or transport is more likely to lead to more confined outbreaks.

6. Microbiological investigations in food and the environment

Sample selection and preparation of seeds

A sample size of 50 g (rather than the 25 g required for other matrices) is recommended to increase the sensitivity of the subsequent diagnostic testing (ANSES, 2011). The seeds are ground then suspended in buffered peptone water and incubated at 37°C for 24 h. 5 ml of the suspension (including some ground seed debris) are vortexed to loosen bacteria adhering to the seed debris. The suspension is centrifuged and then the supernatant is used for DNA extraction (ANSES, 2011).

Detection and identification of STEC O104:H4

The EU Reference Laboratory (EU-RL) for *E. coli* has proposed a method for the detection and identification of STEC O104:H4 in food by Real Time PCR[28]. The method aims at extending the scope of the CEN/ISO Technical Specification 13136 for the detection, in food and feed, of STEC belonging to the 5 serogroups most commonly involved in severe human disease (O157, O103, O111, O145, O26).

According to the approach described in the CEN/ISO TS 13136, the sample is subjected to the Real-Time PCR screening for the presence of the *stx* and *eae* genes followed by, in the *stx*-positive and *eae*-negative samples, the detection of the O104 and H4 antigen-associated genes. Finally, a confirmation step aiming at isolating the STEC strain responsible for the positive PCR reactions is performed. It is noted that if a sample is co-infected with an *eae* positive strain and O104, the latter would be missed by not testing for it. The Real Time PCR screening is performed on DNA extracted from enrichment cultures obtained by incubating a portion of the food sample in an appropriate medium, as described in the CEN/ISO TS 13136. The antimicrobial resistance characteristics of the outbreak strain are exploited for the isolation by plating PCR-positive enrichment cultures onto media supplemented with antibiotics. The suspected colonies are tested for *stx* genes and then for the serotype-specific genes *wzxO104* and *fliCH4*.

[27] RASFF: http://ec.europa.eu/food/food/rapidalert/index_en.htm
[28] http://www.iss.it/vtec/work/cont.php?id=152&lang=2&tipo=3

Microbiological investigations

After identification of Establishment A and sprouting seeds as the food vehicle for the outbreak, further investigations were carried out to identify where STEC O104:H4 may have been introduced into the production and/or distribution chain (the cause). This included studies to assess whether the contamination could have come from the environment (esp. through water), the seeds used for sprouting, personnel or by cross-contamination. Microbiological analysis of the water has thus far proved negative (BfR, 2011a). Several batches of the different seed varieties that were used by Establishment A and were still present at the production site were sampled for microbiological analysis, but none were positive for STEC O104:H4. Exhaustive inspection of all parts of the supply chain showed no likely source of infection after the sprouting at Establishment A.

A recent overview of investigations carried out at MS level and transmitted through the RASFF, shows that a total of 10392 samples were tested, 41 samples were found to be *stx* positive, and 8 STEC O104 positive (*i.e.* 5 from food, 1 from irrigation and processing water, and 2 from the environment, see Appendix I). Thus far *E. coli* O104:H4 has not been isolated from foods where cross-contamination from cases or infected household members could not be excluded. In total, there have been 5 reported isolations from foods, from locations linked to cases. One was from a small remaining part of a cucumber which had been collected from a communal organic waste bin. A second sample was from leftover seed sprouts (one of the mixtures with fenugreek seeds produced by Establishment A) in an open package that was collected from a communal waste bin. In both cases, the vegetable leftovers were taken by household members out of the waste bins. A third sample was from a bell pepper. The other 2 samples were from raw and cooked salmon. These last 3 samples were all obviously contaminated during the incubation period in the laboratory carrying out the analysis (personal communication).

7. Discussion

7.1. Public health aspects

STEC strains with similarities to the outbreak strain (serotype O104:H4, Stx2 production, positive for the genetic markers of enteroaggregative adhesion) have been sporadically isolated in Europe since 2001. The O104:H4 outbreak strain is a typical EAggEC strain that has acquired bacteriophages encoding for Shiga toxin (Scheutz et al., 2011; Rasko et al., 2011). If it proves to be of human origin, as for the other EAggEC, it is unlikely that it would have maintained itself in the EU population for such a long time without being recognised as causing severe human health consequences. Instead, it has appeared causing sporadic cases typically with a recent travel history outside the EU. Therefore, we could assume that the O104:H4 clone, during the intervals between the sporadic cases, has maintained itself outside the EU. If it is a pathogen with a human reservoir (rather than zoonotic), it is conceivable that such a reservoir may be present in a population where the acquired immunity towards EAggEC might limit the severity of the clinical manifestation of the infection, and/or the surveillance systems in place may not be adequate to reveal the disease prevalence. If this is correct, the strain may be imported into the EU by returning travellers or migrants, resulting in a few sporadic cases. The Finnish and Italian cases being linked with North Africa support this hypothesis. However, the zoonotic potential of this strain has yet to be characterized, so it remains possible that reservoirs of this pathogen could be maintained in both wild and domestic animals, including those within the EU.

This outbreak, originating from contaminated seeds rather than, sporadic individual cases, allowed the agent to enter into the food chain and resulted in significant exposure and a major outbreak, which had not been the case for previous sporadic cases reported in the EU. While the epidemiological evidence strongly points to contaminated sprouting seeds as the main vehicle for the outbreaks observed in Germany and in France, as well as the related cases observed outside Germany, but with previous recent travel to Germany; the most recent cases observed in Germany and in other countries may result from vehicles or modes of transmission other than directly from contaminated seeds and sprouts thereof. Since in Germany thousands of people were infected, it is not surprising that secondary cases occur (Hauri et al., 2011; Kuijper et al., 2011) (see also Aldabe et al., 2011, for reporting on household transmission in the French outbreak), or even secondary clusters when infected food handlers are involved (see Grieg et al., 2007 for a review of outbreaks where food handlers are implicated). In addition, it is well known that enteric pathogens for which there is a high risk of infection from low levels of exposure cause outbreaks with person-to-person transmission, as occurs for shigellosis and, for secondary cases, for STEC O157 (Snedeker et al., 2009).

Given the considerable number of asymptomatic carriers, person-to-person transmission is likely to play a role in the spread of the outbreak, resulting in the recognition of apparently sporadic cases without any identified link to a known case, vehicle or cluster. This mode of transmission may result in outbreaks in institutions such as day-care centres, schools or nursing homes.

The limited information available on the infectivity makes it difficult to quantify the role that person-to-person transmission may play in the future. From a bacteriological perspective, a key issue that needs to be considered is that the outbreak strain is an enteroaggregative *E. coli* that produces Stx, and not a "true" STEC. However, EAggEC are also known to have caused foodborne outbreaks in Europe: links with restaurants have been frequently reported as well as a possible role of food handlers, as in a recent outbreak in Italy (Scavia et al., 2008).

In addition, if such asymptomatic food handlers contaminate food, as probably happened in a school in Germany, new clusters may appear with different vehicles of infection[29].

In summary, following a dramatic decrease in the number of new cases during 28 June – 7 July, new cases and clusters continued to occur despite the identification of the original vehicle (sprouting seeds)

[29] http://www.kreis-paderborn.org/kreis_paderborn/presse/2011/entries/erster-todesfall-ehec.php

and the communication of public health advice to avoid consuming sprouted seed. New cases and clusters were most probably due to secondary STEC infections, through different vehicles or by person-to-person transmission. The emerging evidence of a substantial proportion of subclinical infections is important as they are generally unrecognised and unreported, and may contribute to further transmission of the infection. This may have an impact on the epidemiology of the disease and should be taken into account when defining public health measures to prevent further spread.

Regarding seeds already imported, trace-forward activities were advised. During the period that these activities are under way, the advice to consumers not to grow sprouted seeds for consumption and not to eat sprouts or sprouted seeds, unless they have been cooked thoroughly[30], was maintained. Once completed the trace forward operation, and the implicated lot(s) removed from the market, such advice should be revised.

7.2. Epidemiological aspects

The investigations conducted thus far have demonstrated that there was a single hazard (STEC O104:H4) involved in the outbreak. In Germany, the clusters were all linked to sprouted seeds produced by one establishment. The tracing back demonstrated a probable common source for the German and French outbreaks, a particular lot of fenugreek seeds imported from Egypt.

The cause of the outbreak i.e. how, when and where the seeds became contaminated has not (yet) been demonstrated. Assuming this took place during the primary production process, this part of the investigation would need to extend beyond the point of EU import to include the site(s) of production.

7.3. Microbiological aspects

The inability thus far to demonstrate the presence of E. coli O104:H4 in the suspected seeds is not unexpected. It is, of course, possible that contaminated seeds were no longer in stock when sampling took place. However, it is important to realise that a negative laboratory test does not prove the absence of a pathogen. This is because sample-based sampling always involves some statistical uncertainty, where there is a risk for not collecting samples of the contaminated lots or part of a lot that is contaminated. This risk increases when the contamination occurs at a very low prevalence (*i.e.* few units are contaminated). Besides the statistical uncertainty, the test sensitivity plays a role for detecting a positive sample. The test sensitivity is a measure of how good a detection method is to detect microorganisms in a contaminated sample. The rule is that no method is 100% effective. So even though samples of contaminated material have been collected, it may be that the amount of pathogens in the sample is so low that the diagnostic method is unable to detect them.

The uncertainties described above may be particularly true when dealing with seeds, because the matrix is made up of particles, which may become singularly contaminated and dispersed in lots of large dimensions. In addition, the contamination may be heterogeneously distributed within the food matrix as well as, and maybe as a result of, varying physico-chemical conditions for supporting the survival or growth of pathogens. Previous studies have shown enterobacteriaceae to be present on the tissue surface of plants as well as internalised within various the plant tissues including seeds. This may happen at primary production, through irrigating with contaminated water or application of untreated or insufficiently treated fertilizer still containing enteric pathogens) (EFSA, 2011a).

The difficulties of detecting STEC in seeds and sprouts thereof with the applied sampling plans and analytical methods when the contamination is low and unevenly distributed have been observed in

[30] http://www.efsa.europa.eu/en/topics/topic/ecolioutbreak2011.htm

previous outbreaks. For example, in an outbreak of *Salmonella enterica* serotype Enteritidis phage type 4b associated with bean sprouts in the Netherlands in 2000 (Van Duynhoven et al., 2002), it was noted that the outbreak occurred even though at the implicated production facility, every new batch of seeds and samples from each batch of sprouted seeds harvested each day was routinely tested for *Salmonella* spp.

The proposed molecular analytical method is characterised by a high sensitivity. However, as seeds are generally contaminated at very low level (0.1 to 1.8 cfu/g as previously assessed for *Salmonella* (Liao and Fett, 2003)), test portions would then need to weigh more than the recommended 50g, which may not be the case for leftover samples collected during outbreak investigation. In addition, bacteria on or in seeds may undergo stress induced by very low water activity and therefore exhibit low culturability during the enrichment phase which precedes the PCR step (*i.e.* viable but non-culturable (VBNC) state).

Furthermore, seeds, as a biological matrix, present particular characteristics (*e.g.* very low water activity, biofilm etc.) which must be considered when defining the analytical phases preceding the PCR step. The method proposed by the EU-RL includes an annex specific for seed testing. However, it has been recently suggested, elaborating from methods typically used in phytopathology for testing for bacterial pathogens from seeds, to perform a preliminary step of seed soaking, to facilitate the revivification of stressed bacteria (ANSES, 2011). Further investigations are still needed to determine an optimal test portion of seeds, fully define preliminary analytical steps and assess the impact on the sensitivity of the test method. Potential growth and PCR-inhibitory effects of the seeds may also have to be taken into account.

Work has been initiated by the EU-RL to evaluate the test methods for seeds in use by coordinating a dedicated working group and organising an inter-laboratory study on seeds naturally contaminated with STEC other than O104. Seed lots naturally contaminated with STEC represent a valuable material for conducting studies to evaluate and validate specific laboratory methods. Indeed, it is very difficult to define spiking strategies that could reliably mimic the contamination naturally occurring in field conditions.

According to the protocol for seed testing proposed by the EU-RL, the reporting of a negative result or a presumptive positive result after the first screening step (detection of *stx* genes) will require 26-28 hours. The isolation of STEC O104:H4 from PCR-positive enrichment samples could require 48-72 hours. It is also possible that the positive results obtained in the PCR screening step, both for STEC in general or specifically for STEC O104, are not confirmed by the isolation of the STEC strain. The meaning of such a result will depend on the epidemiological setting driving the sampling: in a crisis setting and with samples presumably related to an outbreak.

CONCLUSIONS

Description of the outbreak

Initial epidemiological studies of the German outbreaks indicated fresh salad vegetables as the probable vehicle of infection. Further, more detailed studies implicated fresh sprouting seeds.

Tracing forward and backward the dissemination of the implicated sprouting seeds demonstrated that all of the clusters for which there was sufficient information, could be linked to a single sprouted seed producer in Germany.

No evidence for environmental contamination was found at the premises of the sprouted seed producer. Employees found to be infected were not thought to be the origin of the contamination due to the comparative dates of onset of illness with those of consumers. As a consequence, and taking into account experience from other outbreaks of enteric pathogens through consumption of contaminated sprouting seeds, contaminated seeds were hypothesized as being the most likely source. Fenugreek sprouts were mostly sold as mixtures of sprouts, so that at this stage, it was not possible to identify the specific sprout (and seed) type responsible.

The STEC strain responsible (O104:H4) for both the German and French outbreaks was found to be indistinguishable, strongly indicating that there was a common source for both outbreaks.

The comparison of the back tracing information from the French and German outbreaks led to the conclusion that a specific lot of fenugreek seeds imported from Egypt was the most likely source of both outbreaks, although it could not be excluded that other lots imported by the same importer from the same exporter may be implicated.

The cause of the outbreak, *i.e.* how, when and where the seeds became contaminated has not (yet) been demonstrated. Assuming this took place during the primary production process, this part of the investigation would need to extend beyond the point of EU import to include the site(s) of production.

Prior to the 2011 outbreak, STEC O104 was a very rare serogroup in humans in the EU and worldwide. Sporadic cases in the EU have been linked to travel to North Africa, the Middle East and Central Asia.

Implementation of optimised testing of seeds and sprouted seeds

Work has been initiated by the EU-RL to evaluate the test methods for seeds in use by coordinating a dedicated working group and organising an inter-laboratory study.

Prevention of seed contamination during production and distribution

The preparation of fresh sprouted seeds does not always include a step where pathogen contamination is eliminated. The consumption of fresh sprouted seeds however, is based on the understanding that they are sold as ready to eat. For fresh produce this assumes and relies on a production process which prevents contamination and ability to detect contamination when it occurs. These conditions have proven not to have been met in the case of the 2011 outbreaks.

Previous food-borne outbreaks due to sprouting seeds have led public authorities in some countries (FSANZ, 2010) to carry out risk analyses to identify critical steps and effective measures in the

production and processing of sprouted seeds to prevent contamination of the food chain (EFSA-Q-2011-00877[31]).

Prevention of further exposure to the lots of contaminated seeds and sprouts thereof

In the short term all efforts were focused on the prevention of any further exposure of the consumer to sprouts of seeds from the lots of concern. EFSA and ECDC strongly recommended advising consumers not to grow sprouted seed for their own consumption and not to eat sprouted seeds unless they have been cooked thoroughly (ECDC and EFSA, 2011).

Furthermore, it was important that a trace back investigation be initiated on the incriminated lot(s) of fenugreek seeds in the country from which they were exported to the EU. In addition, Member States and third countries were encouraged to initiate or complete forward tracing of companies receiving the suspect lot(s).

Prevention of secondary infections via food and through person-to-person transmission

To complement public health measures regarding food handling, information to the public should stress the need for proper hand washing, in addition to the specific advice related to sprouted seeds.

Since there is evidence of asymptomatic carriers of STEC in humans, screening of humans involved in food handling is relevant. The monitoring and/or exclusion of STEC carriers from food handling should be considered as a mitigation option.

It is extremely important to further strengthen epidemiologic surveillance of human cases. Germany and France have developed protocols to thoroughly investigate the likely mode of transmission of new cases. The ECDC will be compiling results from these investigations using the EPIS platform[32].

[31] http://registerofquestions.efsa.europa.eu/roqFrontend/?wicket:interface=:1::::
[32] http://www.ecdc.europa.eu/en/activities/epidemicintelligence/Pages/Activities_EpidemicIntelligence.aspx

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APPENDIX I

RASFF news – notification – Food – Information Exchange – 11-658-add17. Subject: EHEC outbreak: overview of investigations.

GLOSSARY

EAggEC – Enteroaggregative *Escherichia coli*

EHEC – Enterohaemorrhagic *Escherichia coli*

ESBL – Extended-spectrum beta-latamase

EU-RL – European Union Reference Laboratory

HUS – Hemolytic-uremic syndrome

PCR – Polymerase chain reaction

RASFF – Rapid Alert System for Food and Feed

STEC – Shiga toxin-producing *Escherichia coli* (synonym for VTEC)

VTEC – Vero cytotoxin-producing *Escherichia coli* (synonym for STEC)