An international outbreak of Shiga toxin-producing *Escherichia coli* O157 infection due to lettuce, September – October 2007

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Between 14 September and 20 October 2007, an outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157 simultaneously occurred in the Netherlands and Iceland. A total of 50 laboratory-confirmed cases were reported with a STEC O157 infection caused by the same clone. The strain was of type O157:H-, PT8, positive for stx_1, stx_2, *eae* and *e-hly*, and sorbitol negative. The most probable cause of this international outbreak was contaminated lettuce, shredded and pre-packed in a Dutch food processing plant. Samples of the environment, raw produce and end products, taken at several vegetable growers and processing plants all tested negative for STEC O157. However, the only epidemiological link between the cases in the Netherlands and in Iceland was the implicated Dutch processing plant. In Europe, food products are often widely distributed posing the risk of potential spread of food borne pathogens simultaneously to several countries. This international outbreak emphasises the importance of common alert and surveillance systems in earlier detection of international outbreaks and better assessment of their spread.

Introduction

Infections with Shiga toxin-producing *Escherichia coli* (STEC) typically present as diarrhoea which can range from mild and watery to bloody (haemorrhagic colitis). The infection can progress to haemolytic uremic syndrome (HUS), a serious condition that can result in death [1,2]. Children under 10 years of age with a verified STEC infection have approximately 15% risk of developing HUS [3]. The serotype most often associated with severe disease is STEC O157:H7, but many other serotypes are also known to cause symptoms [4-6].

A great majority of STEC O157 outbreaks can be traced back to ruminants, especially cattle [7]. Numerous studies have been done on faecal excretion of STEC O157 from cattle to estimate the carriage rate of STEC [7]. All agree that faecal excretion exists, although the rate found varies. Inevitably, contact with farm animals has been reported as a source for STEC outbreaks [8,9]. Spreading of cattle manure over land or in water can contaminate water and produce, and meat can be contaminated in the slaughterhouse or later in the production process. Water [10,11] and food products [12], such as meat [13-15], dairy products [16-18], and fresh produce [19,20] are therefore often reported as sources of outbreaks caused by STEC O157.

In September-October 2007, national outbreaks of STEC O157 infection occurred simultaneously in the Netherlands and Iceland, of which preliminary reports were published in November 2007 [21,22]. As the isolates of STEC O157 from the patients of both outbreaks had an identical and unique pulsed-field gel electrophoresis (PFGE) pattern, a common source was suspected. In the present report, we have combined the results of the outbreak investigations done in both countries into one description of the international outbreak, with lettuce as the most probable cause.

Methods

The Netherlands

Since 1999, an enhanced laboratory-based surveillance of STEC infections has been implemented in the Netherlands. This means that all Dutch medical microbiological laboratories are required to send STEC isolates to the National Institute for Public Health and the Environment (RIVM) for O- and H-typing. The isolates are also tested for genes encoding Shiga toxin type 1 and type 2 (stx_1 and stx_2), the *E. coli* attaching-and-effacing gene (*eae*) and the haemolysin encoding EHEC-*hly* gene (*e-hly*). DNA fingerprints are made by PFGE, using *Xba*I as the restriction enzyme. The fingerprints are processed with BioNumerics® (Applied Maths, Kortrijk, Belgium; Dendrogram type=UPGMA, Similarity coefficient=Dice).

Additionally, as part of the surveillance, municipal health services contact every laboratory-confirmed STEC patient in the Netherlands to collect information about clinical symptoms and exposures to known risk factors in the week before illness onset using a standardised questionnaire [23,24]. When a marked increase in the numbers of reported STEC cases was observed in the end of September 2007, in addition to the standard questionnaire a special outbreak questionnaire was designed, providing more detailed information on consumption of meat, dairy and raw

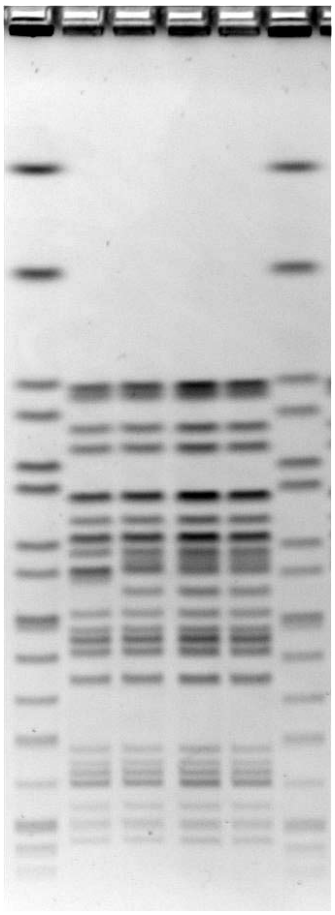
vegetables and contact with farm animals and manure. All cases with onset of symptoms after 1 September 2007 were asked to complete both questionnaires.

An outbreak-related case was defined as having an isolate matching the outbreak fingerprint for at least 95% and the date of onset of symptoms later than 1 September 2007. A case-case comparison was made between non-outbreak cases of the enhanced surveillance (1999-2007) and the outbreak-related cases using the standardised questionnaire.

When lettuce was suggested as a possible source, the Dutch Food and Consumer Product Safety Authority (VWA) started investigating the distribution channels of packed fresh vegetables and the individual ingredients, and visited several vegetable producing and processing companies. During these visits, samples of the environment, raw produce and end products were collected and tested for STEC O157. During the visit of VWA at one of the processing plants, it was noted that during the outbreak period a high number of workers had been absent due to illness. The municipal health service visited the plant shortly afterwards to gather additional information on the symptoms and to collect stool and blood samples from those who had been ill. Blood samples were tested for the presence for antibodies against LPS O157 using ELISA and immunoblotting [25].

FIGURE 1

Pulsed-field gel electrophoresis (PFGE) pattern of the outbreak strain (middle four lanes) and the reference H9812 *S. Braenderup* (both side-lanes), international outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157 in Iceland and the Netherlands, September-October 2007



Iceland

In Iceland, STEC infections are subject to mandatory notification which requires laboratories and treating physicians to report cases without delay. When a clear rise in the number of domestically acquired STEC O157 infections was observed in early October 2007, an outbreak investigation was initiated.

The case definition used for the outbreak investigation included all domestically acquired STEC O157 infections with onset of symptoms after 1 September 2007, pending PFGE and testing for stx_1 and stx_2 genes. A trawling questionnaire on food consumption, mass gathering and travel as well as purchase records from two weeks prior to onset of infection were collected from cases.

In Iceland, detection of STEC O157 is carried out only by the reference laboratory which performs DNA fingerprinting with PFGE, using *Xba*I as the restriction enzyme. Testing for stx_1 and stx_2, however, is not done in Iceland therefore isolates were sent to the Laboratory of Enteric Pathogens at the Health Protection Agency (HPA) in the United Kingdom for detection of these genes.

The food division of the Environment and Food Agency in Iceland is responsible for surveillance in food and when results from the trawling questionnaire and purchase records were available, surveillance of lettuce was intensified with increased sampling.

Results

On 11 October 2007, Iceland notified other European countries about the ongoing outbreak of STEC O157 through the urgent inquiries system of the European Food and Waterborne Diseases Network administered by the European Centre for Disease Prevention and Control (ECDC). When the Netherlands responded by reporting a similar outbreak, contact between these two countries was established and information exchange was facilitated. PFGE patterns of the first set of STEC isolates from cases in both countries were available for comparison on 22 October revealing identical fingerprints and a definite link between the two countries. The PFGE-pattern is shown in Figure 1.

In total, isolates from 48 cases from both countries had identical PFGE patterns. This pattern had not been observed previously in either of the two countries or the rest of Europe. Isolates from two other individuals generated a PFGE pattern that matched the outbreak pattern in 95-97%. Both patients were included as cases, resulting in a total number of 50 cases. The distribution of these cases by date of symptom onset is shown in Figure 2. Forty-seven cases (94%) reported diarrhoea, including 41(87%) with bloody diarrhoea. No cases of HUS were reported.

Twelve cases, seven males and five females, were regarded as secondary cases, as they most likely had contracted the infection from another case. Six of them were children aged 0-8 years, and six were adults aged 34-82 years.

The 38 primary cases included 21 females and 17 males. Their median age was 24.5 years (range 1-74 years), and about half of

the cases were aged between 10 and 30 years. The detailed age and sex distribution of the primary cases is shown in Figure 3.

The Netherlands

In the Netherlands, the annual number of STEC cases reported between 1999 and 2006 ranged from 32 to 57. In the end of September 2007, a marked increase in the number of reported cases was noted. An outbreak of STEC O157 was identified including 41 cases with dates of symptom onset between 14 September and 20 October 2007, of whom 31 were primary cases, and ten were secondary cases. Thirteen patients were admitted to hospital; in two cases information on hospitalisation was missing. All 41 isolates were of serotype O157:H-, contained stx_1, stx_2, *eae* and *e-hly* genes, and were sorbitol negative.

Answers to at least one of the two questionnaires were available for 29 of the 31 primary cases. Descriptive epidemiology suggested a link between STEC infection and consumption of lettuce as 25 cases (86%) reported eating lettuce in the week before illness. Comparison of the standard questionnaire results for the outbreak-cases with those of the sporadic cases of the surveillance showed highest odds ratios for pre-packed lettuces: 4.41 (95% confidence interval 1.91-10.19) compared to the sporadic cases of 1999-2007, and 7.33 (95% CI 2.19-24.50) compared to the sporadic cases of 2007.

A total of 99 environmental and food samples taken at the vegetable producing and processing plants were tested and found negative for STEC O157. In one company, which exported pre-packed lettuce to Iceland, a total of 32 employees had been on sick leaves because of gastroenteritis during the outbreak period. However, faeces and blood samples of these workers tested negative for STEC O157 and interviews with them suggested that the clinical presentation was more compatible with a norovirus outbreak than with STEC O157 infection.

Iceland

In Iceland, only up to two cases of STEC infection had been reported annually in the 10-year period preceding 2007 (with the exception of four cases notified in 2004), and no outbreak had ever been detected. In the outbreak in 2007, nine cases were identified with onset of symptoms between 23 September and 18 October. Seven cases were considered primary cases and two were secondary cases. Seven patients were hospitalised.

The isolates from the first three cases were sent to the Laboratory of Enteric Pathogens at the HPA in the United Kingdom and were identified as STEC O157, phage type 8, carrying the stx_1 and stx_2 genes with a PFGE pattern identical to the pattern for the Dutch strains. PFGE done on all nine isolates at the Department of Microbiology at Landspitali University hospital revealed a pattern identical to the pattern from HPA.

The seven primary cases lived in different parts of the country: three cases resided in the capital area, two in the northern part of the country, one in the eastern part and one on the Westman Islands. It was clear that the product that had caused the infection had been widely distributed.

Eight cases (seven primary) answered the trawling questionnaire and two primary cases provided purchase records. Results from the outbreak questionnaire showed that six of the seven primary cases had consumed either fish or sliced precooked ham. But since these products originated from different producers or local fishermen, they were considered to be an unlikely source of the outbreak. The purchase records and the outbreak questionnaire also revealed that five of the seven primary cases had consumed ready-to-eat lettuce mixtures of one brand pre-packed in and imported from the Netherlands. However, of the 80 samples of lettuce collected between 22 October and 5 November none tested positive for *E. coli* O157.

Discussion

Between mid-September and mid-October 2007, in Iceland and the Netherlands a total of 50 patients were diagnosed with a STEC O157 infection caused by the same strain. The actual number of cases may have been considerably higher seeing that infections with STEC O157 may pass uncomplicated or even symptom-free, especially in adults [26,27], and those affected do not seek medical help and are not tested for STEC O157. No HUS-cases have been reported in the outbreak. The age of the cases is probably a relevant indication of the cause, as only three of the 38 primary cases and five of the 12 secondary cases were younger than five years.

Figure 2

Epidemic curve of the international outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157 in Iceland and the Netherlands, September-October 2007, by date of onset of symptoms (n=50)

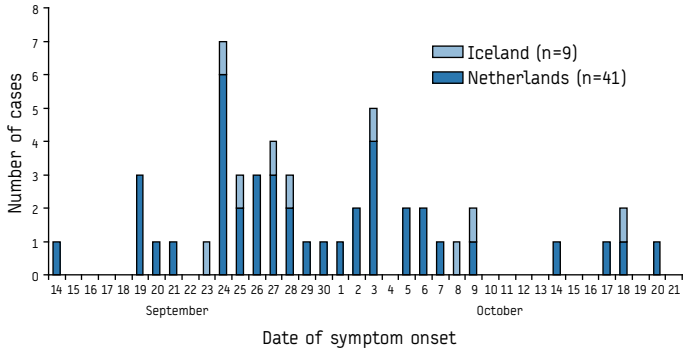
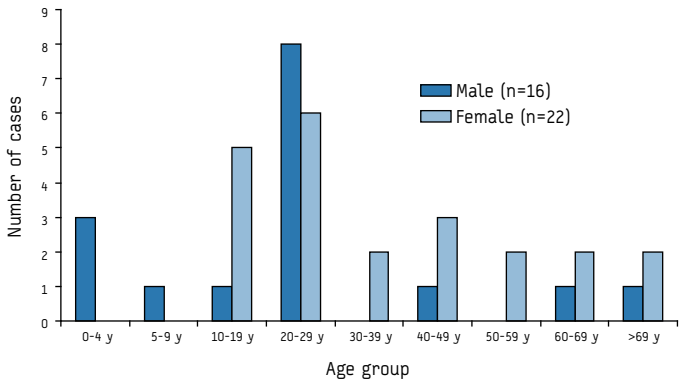


Figure 3

Age and sex distribution of all primary cases of Shiga toxin-producing *Escherichia coli* (STEC) O157 related to the international outbreak in Iceland and the Netherlands, September-October 2007 (n=38)



The most probable cause of this international outbreak was contaminated lettuce, shredded and pre-packed in a Dutch food processing plant. Packages with several combinations of different types of lettuce but belonging to the same brand imported from the Netherlands were reported by the cases. Contamination of lettuce can occur either during growth by the application of water, soil or manure contaminated with animal faeces or as a result of cross-contamination during processing, for example through contaminated transport containers, human transmission or in the shredding process. However, microbiological evidence pointing to the source of this outbreak was not found. Furthermore, none of the workers of the implicated food processing plant tested positive for STEC O157 infection. It is likely that the contamination had already faded out at the time samples were taken at the food producing and processing plants and that contaminated products had not been present in the supermarkets anymore. The sampling started in mid-October, which is around the date the last cases had onset of symptoms.

The outbreak highlights the importance of fresh produce as a vehicle in STEC infections. Although it has been shown that lettuce can become infiltrated by *E. coli* O157 making it impossible to wash off [28], in most cases the bacteria stay on the surface of the leafs. However, the fact that salad vegetables are usually eaten raw is compounded by the increase in popularity of pre-packed salad products that are unlikely to be washed by the consumer. In one outbreak caused by lettuce, the wash water used by the grower was the most likely source of contamination, as it contained *E. coli* O157:H7 [29]. Contaminated water was also suspected as the source in an STEC O157 outbreak related to iceberg lettuce in Sweden, although no microbiological evidence was found [30]. The trace-back investigation in another lettuce outbreak in the United States implicated two possible sources: one at a local farm and another in six farms shipping under the same label [31]. Microbiological evidence could not be established, so the transmission route remained unclear.

Food products are widely distributed within the European Union (EU) and from and to countries outside the EU thus creating the potential for the spread of food borne pathogens simultaneously to several countries. This international outbreak emphasises the importance of common alert and surveillance systems in the EU for earlier detection of international outbreaks and better assessment of the size and the spread of such outbreaks. The e-mail urgent inquiries system of the European Food and Waterborne Diseases Network administered by the ECDC has proven its value to detect similar outbreaks occurring simultaneously in more than one country. In this outbreak, the link to Dutch lettuce products was suspected two weeks after the first e-mail informing about the cases in Iceland. As both countries promptly joined forces, direct action by the Dutch food authorities could then be taken, which shows the added value in joint outbreak investigation within the EU. Analysing compiled data when possible and collecting supporting findings from more than one country, at the same time increases the possibility to detect potential sources at an earlier stage and strengthens the epidemiological evidence. Thus, cooperation allows for earlier implementation of actions aimed at identifying and eliminating the source of infections and therefore contributes to the decrease of both morbidity and mortality due to communicable diseases within the EU.

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