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Shiga toxin-producing *Escherichia coli* (STEC) O157 outbreak, The Netherlands, September – October 2005

Y Doorduyn¹, CM de Jager¹, WK van der Zwaluw², IHM Friesema¹, AE Heuvelink³, E de Boer³, WJB Wannet², YTHP van Duynhoven¹

1. Centre for Infectious Diseases Epidemiology, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
2. Diagnostic Laboratory for Infectious Diseases Diagnosis and Perinatal Screening, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
3. Food and Consumer Product Safety Authority, Zutphen, The Netherlands

In September 2005, the first national food-related outbreak of Shiga toxin (Stx)-producing *Escherichia coli* (STEC) O157 was investigated in the Netherlands. A total of 21 laboratory-confirmed cases (including one secondary case), and another 11 probable cases (two primary and nine secondary cases) were reported in patients who became ill between 11 September and 10 October 2005. Preliminary investigation suggested consumption of a raw beef product, steak tartare (in the Netherlands also known as 'filet américain'), and contact with other symptomatic persons as possible risk factors. A subsequent case-control study supported the hypothesis that steak tartare was the source of the outbreak (matched odds ratio (OR) 272, 95% confidence interval (CI) 3 - 23211). Consumption of ready-to-eat vegetables was also associated with STEC O157 infection (matched OR 24, 95% CI 1.1 – 528), but was considered a less likely source, as only 40% of the cases were exposed. Samples of steak tartare collected from one chain of supermarkets where it is likely that most patients (67%) bought steak tartare, all tested negative for STEC O157. However, sampling was done three days after the date of symptom onset of the last reported case. Since 88% of the cases became ill within a two week period, point source contamination may explain these negative results. It is concluded that steak tartare was the most likely cause of the first national food-related outbreak of STEC O157 in the Netherlands.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) O157 infection is the leading cause of haemorrhagic colitis and haemolytic uraemic syndrome (HUS) in children [1]. In adults, it mainly causes uncomplicated bloody diarrhoea. Well-known vehicles for transmission of STEC O157 include contaminated food products, especially of bovine origin, such as milk and undercooked beef [2-4], but also fresh produce, such as raw fruit and vegetables [5-7]. Other modes of transmission are person-to-person spread, contact with animals or their manure, and contact with contaminated water [4, 8-9].

Since January 1999, the Netherlands has implemented enhanced surveillance of STEC O157 [10]. Notification of STEC O157 infections became mandatory in December 1999. Since then, between 35 and 57 symptomatic cases were reported annually, corresponding to an incidence of about 0.22 to 0.35 laboratory-confirmed cases per 100 000 inhabitants. Although molecular typing and epidemiological information regularly suggested small clusters of fewer than 5 cases, large clusters or outbreaks had not previously been identified [11]. In the first week of October 2005, 18 cases were reported. This high number of cases was unprecedented and an outbreak was suspected. This paper describes the subsequent outbreak investigation.

Methods

As part of the enhanced surveillance, all Dutch laboratories are requested to report positive results of STEC O157 to the local public health service. Furthermore, they are requested to send the STEC O157 isolates to the National Institute for Public Health and the Environment (RIVM) for O- and H-serotyping, for testing for genes encoding Shiga toxin 1 (stx1) and 2 (stx2), *Escherichia coli* attaching and effacing (eae) gene and the enterohaemorrhagic

Escherichia coli haemolysin (e-hly) gene by polymerase chain reaction. DNA fingerprints are made by pulsed-field gel electrophoresis (PFGE), using XbaI as the restriction enzyme. For the current outbreak, BlnI was used as a second restriction enzyme. The fingerprints are processed using Bionumerics software (Applied Maths, Belgium). In addition, for the current outbreak, 15 isolates were sent to the Health Protection Agency's Laboratory of Enteric Pathogens in London for phage typing. The local public health services are requested to contact every reported patient to collect background information using a standardised questionnaire. The questionnaire includes questions about clinical manifestation, exposures in the seven days before symptom onset, such as contact with symptomatic individuals (within or outside the household), travel, food consumption such as beef, pork, poultry, vegetables, fruit, and dairy products), eating in a restaurant, contact with farm animals or manure, water-related activities, and working or playing in the garden. All questionnaires are returned to the RIVM. For further details see [10].

Within the first week of October 2005, an unusual high number of 18 cases was reported. This triggered interviews with 11 of these cases, using a trawling questionnaire to generate hypotheses about possible sources. From these interviews, consumption of steak tartare and contact with other persons with gastroenteritis symptoms emerged as possible risk factors. A case-control study was started on October 10 to test the hypothesis that steak tartare was the source of the outbreak.

We defined a confirmed case as a person with diarrhoea (= 3 loose stools within 24 hours) with two or more additional symptoms (nausea, abdominal pain, abdominal cramps, blood in stool, mucus in stool, vomiting or fever) after 1 September 2005, with a stool specimen positive for STEC O157 and a PFGE pattern matching the outbreak type. A probable case was defined as a person with diarrhoea after 1 September 2005, and epidemiologically related to a confirmed case (e.g., household contact, friend, school or work contact). For probable cases, no stool specimens were available for testing for STEC O157. Cases could be primary, if the date of symptom onset was earlier than or equal to the symptom onset of a related case, or secondary, if their illness started at least two days later than a related case. Probable cases were included to measure the magnitude of the outbreak, but were excluded from the case-control study.

The local public health services interviewed all confirmed cases using the surveillance questionnaire and an additional outbreak questionnaire to obtain detailed information about contact with symptomatic persons and consumption of beef products (steak tartare, minced beef, mixed beef and pork mince, minced steak, and hamburger) within seven days before symptom onset. Questions were asked about the shops where these products were bought to determine whether any foods shared a common source. For each confirmed case, two controls were recruited using a web-based phone book, matching for neighbourhood (streets in the same area) and age group (0-9, 10-17, 18-49, >50 years). Each fifth phone number in a street was called until two eligible controls were found and willing to participate. Controls were interviewed by telephone using a standardised questionnaire composed of the two questionnaires used for the cases. The questionnaire addressed exposures in the week of 17 September, which was for most cases the week before symptom onset. The controls of the last reported case were interviewed about exposures in the week of 26 September. When a control was 17 years or younger, a parent or guardian was interviewed in his or her place. Univariate and multivariate conditional logistic regression analyses were performed using PROC PHREG in SAS version 9.1. Variables with a P value ≤ 0.15 in the univariate analyses were selected for inclusion in the final multivariate model by (manual) stepwise forward selection. Variables for which the likelihood ratio test gave a P value ≤ 0.05 and variables with a confounding effect (changing the beta-estimates with at least 15%) were kept in the multivariate model.

The Netherlands participates in Enter-net, an international surveillance network for Salmonella and Verocytotoxin-producing Escherichia coli O157 infections, funded by the European Commission [12]. All participating countries were informed about the outbreak and requested to forward information about cases of STEC O157 infection with a similar strain (O-, H-, stx1, stx2 type and PFGE pattern).

On 13 October, the Food and Consumer Product Safety Authority started a national sampling of steak tartare from one chain of supermarkets that was frequently mentioned by the patients. All samples were tested for STEC O157. The agency also interviewed the directors

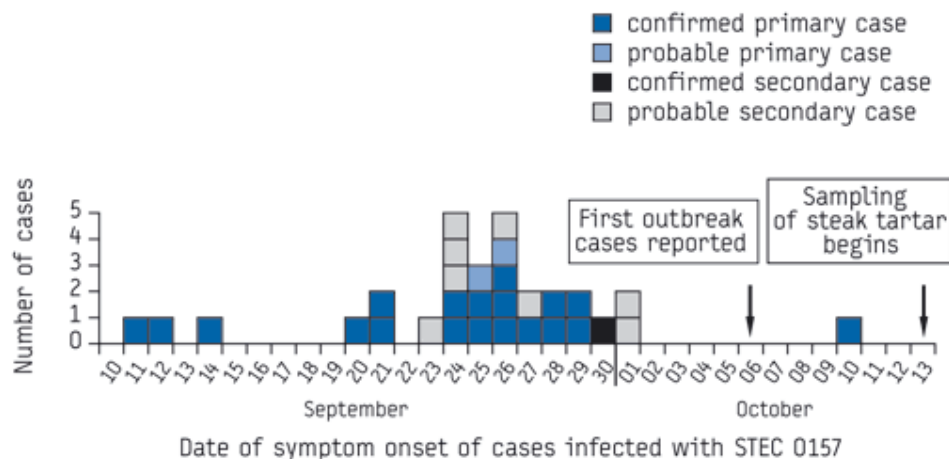
of these supermarkets for details concerning the providers of steak tartare in the week of 17 September.

Results

We identified 21 confirmed cases (of which one was a secondary case) and eleven probable cases (two primary and nine secondary cases), who had dates of symptom onset between 11 September and 10 October [FIGURE 1]. All 15 isolates sent for phage typing showed an identical phage type, RDNC. The median age of the confirmed cases was 24 years (range 3-66 years). Compared with the age distribution of cases in the routine surveillance, a lower proportion of outbreak cases was in children aged 0-4 years (10% versus 27% in the surveillance). Fifty two per cent of cases were female. Cases were distributed throughout the Netherlands. After diarrhoea, the most commonly reported symptoms were abdominal pain (95%), abdominal cramps (95%), blood in the stool (81%), looking pale (71%), listlessness/narcolepsy (67%), nausea (57%) and mucus in the stool (52%). None of the cases developed HUS. Seven patients (33%) were admitted to hospital, and the median length of stay was four days (range 3-7 days). Only confirmed primary cases were included in the risk analysis. Based on the univariate analysis, consumption of steak tartare, ready-to-eat raw vegetables, minced beef, contact with horses and swimming were considered in the multivariate model, but only steak tartare and ready-to-eat raw vegetables remained associated with illness (Table). Seventy five per cent of the patients consumed steak tartare, compared with only 20% of the controls. For ready-to-eat vegetables, these proportions were 40% and 25%, respectively. Of the cases who consumed steak tartare, 67% mentioned a specific supermarket chain as the place where they bought the steak tartare, but many of these cases named a second supermarket or butcher as well. Only one of the eight controls who consumed steak tartare mentioned that supermarket chain.

FIGURE 1

Epidemic curve of 20 confirmed cases and 10 probable cases in an outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157 in the Netherlands, September-October 2005



Note: Symptom onset date for one confirmed primary case and one probable secondary case were unknown: these cases are not included in the figure

The Food and Consumer Product Safety Authority collected 302 samples of steak tartare from this supermarket chain across the Netherlands. All samples tested negative for STEC O157, but *Salmonella* was found in three samples. Trace back led to five possible providers, of which one was most likely to have delivered the steak tartare bought by most patients. Inspection at the site of this provider in the week of 24 October did not reveal anything unusual. Further trace back was not feasible, since the provider obtained meat from many different abattoirs, both nationally and internationally.

TABLE

Matched univariate and multivariate odds ratios of factors associated with the STEC O157 outbreak, the Netherlands, September-October 2005

Exposure	Exposed controls (%)	Exposed cases (%)	Unexposed controls (no.) matched with exposed cases (no.)	Exposed controls (no.) matched with unexposed cases (no.)	Univariate matched OR (95% CI) [†]	Multivariate matched OR (95% CI)
Steak tartare	8 (20)	15 (75)	22 / 15	0 [‡] / 5	19.2 (2.5-149.0)	272.2 (3.2-23211.5)
Ready-to-eat raw vegetables	10 (25)	8 (40)	8 / 8	2 / 12	3.4 (0.6-17.9)	24.2 (1.1-528.3)
Minced beef	26 (65)	7 (35)	6 / 7	18 / 13	0.3 (0.1-1.0)	0.1 (0.0-0.8)
Swimming	9 (23)	8 (40)	12 / 8	5 / 12	2.7 (0.7-9.7)	
Contact with horses	2 (5)	5 (25)	9 / 5	1 / 15	8.6 [#] (1.0-74.9)	

Note: 20 cases matched with 40 controls (1:2) according to age group and neighbourhood

[†] OR, odds ratio; CI, confidence interval

[‡] To be able to calculate odds ratios, one unexposed control matched with an unexposed case was artificially considered as an exposed control in the analysis

[#] Contact took place at different sites, eg. different riding schools and children's farms

In the Dutch surveillance database for STEC O157, two historical cases were found with the outbreak PFGE pattern, whose dates of symptom onset were 12 June and 10 July 2005 [FIGURE 2]. The source of infection of these cases remained unknown. Information from Enter-net participants revealed that no other European countries or the United States had ever identified patients with this PFGE pattern. Since the last reported outbreak case, no new cases with the outbreak strain have been reported.

Discussion and conclusion

This was the first nationwide outbreak of STEC O157 in the Netherlands since the start of the enhanced surveillance. Twenty one confirmed cases were identified, which corresponds with at least several thousand cases in the Dutch community [13,14]. The outbreak was most likely caused by consumption of steak tartare, a beef product that is consumed raw. Because this food is generally known to be a risk product, few young children consume it, explaining the relatively low number of young outbreak cases and the absence of HUS. The second risk factor in the outbreak, ready-to-eat raw vegetables, was considered a less likely source as it could explain fewer cases, and the sales outlets were diverse. Strikingly, subtyping with PFGE showed a unique pattern that was first found in the Netherlands in June 2005 and has not been observed internationally. Phage typing of part of the isolates also showed an unusual type.

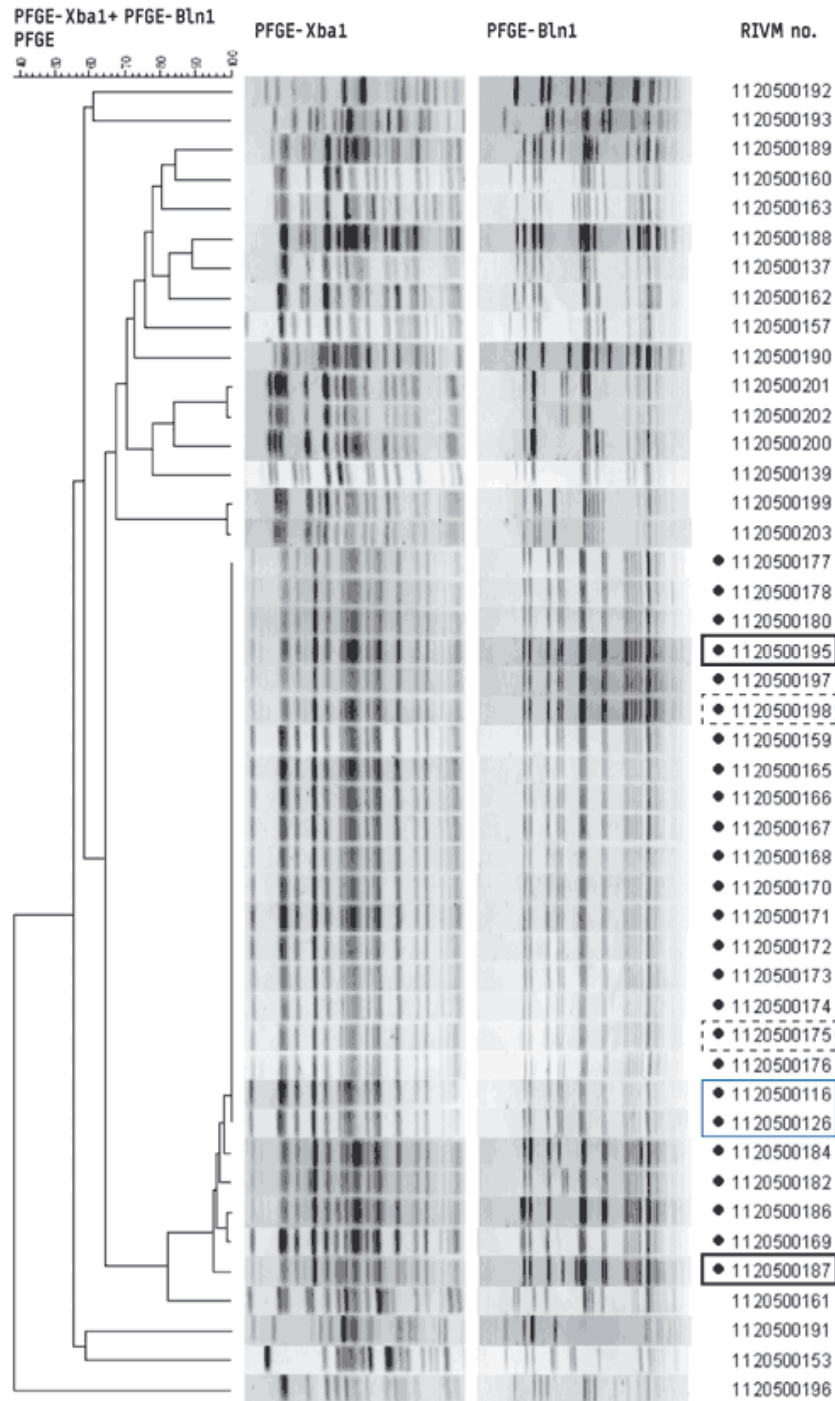
Cattle form the major reservoir of STEC O157 and foods of bovine origin caused many outbreaks of STEC O157 internationally [2-4]. In the Dutch surveillance, consumption of raw or undercooked beef was more frequently reported in 2004 (42%) than in previous years (14% to 23%), when contact with animals or their manure dominated. This may be caused by a change in consumption pattern of the Dutch population or a higher prevalence of STEC at retail due to less hygienic slaughter practices [11]. There is no indication for a higher prevalence of STEC O157 in cattle at the Dutch farms [15], but most of the beef consumed in the Netherlands is imported. It is of interest that several other European countries also experienced national STEC O157 outbreaks at around the same time, [16-18], one of which was also related to a beef product [18].

In the case-control study, controls were interviewed about exposures in the week before symptom onset of most cases, and thus had a similar recall period. A few cases had an earlier date of symptom onset, and therefore they had a longer recall period than their controls.

Although the case-control study clearly indicated steak tartare as the source of infection, samples taken from this product tested negative for STEC O157. However, sampling started on 13 October, one week after the first outbreak cases were reported, while the last outbreak case became ill on 10 October. This suggests that the outbreak may have been caused by a point source contamination of steak tartare. As trace back was incomplete, it could not provide an indication of the level of the food production and processing chain where the STEC O157 contamination was introduced.

FIGURE 2

PFGE patterns of 39 isolates received during enhanced STEC O157 surveillance in 2005 in the Netherlands



- Isolate with the outbreak PFGE pattern (25 isolates of 23 cases shared the outbreak PFGE pattern)
- Two historical cases with date of symptom onset 12 June and 15 July 2005
For these cases PFGE was not done with the second restriction enzyme Bln I
- , - - - - Two isolates of one confirmed case

Because trace back of meat is difficult and time-consuming, and sampling in the relevant period is often not possible, current national monitoring programmes for beef products should be continued. In addition, since other European countries also recently experienced outbreaks of STEC O157 and Salmonella related to beef [18-20], the place of origin of beef should be recorded in these monitoring programmes. To prevent future outbreaks, more attention should be given to hygienic slaughter practices. However, even with improved hygiene in slaughterhouses, pathogens may still be present in raw meat. Therefore, public education is needed to discourage consumption of raw meat products, especially by high risk groups.

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