FOODBORNE PATHOGENS AND DISEASE
Volume 5, Number 3, 2008

DOI: 10.1089/fpd.2007.0065

A Large *Escherichia coli* O157 Outbreak in Sweden Associated with Locally Produced Lettuce

A. Söderström,[1] P. Österberg,[1] A. Lindqvist,[1] B. Jönsson,[2] A. Lindberg,[2] S. Blide Ulander,[3] C. Welinder-Olsson,[4] S. Löfdahl,[5] B. Kaijser,[4] B. De Jong,[5] S. Kühlmann-Berenzon,[5] S. Boqvist,[6] E. Eriksson,[6] E. Szanto,[7] S. Andersson,[8] G. Allestam,[5] I. Hedenström,[5] L. Ledet Muller,[5] and Y. Andersson[5]

Abstract

In 2005 a large outbreak of verotoxin-producing *Escherichia coli* (VTEC) occurred in Sweden. Cases were interviewed and cohort and case–control studies were conducted. Microbiological investigations were performed using polymerase chain reaction (PCR) to detect the Shiga-like toxin (Stx) genes followed by cultivation and pulsed-field gel electrophoresis. A total of 135 cases were recorded, including 11 cases of hemolytic uremic syndrome. The epidemiological investigations implicated lettuce as the most likely source of the outbreak, with an OR of 13.0 (CI 2.94–57.5) in the case–control study. The lettuce was irrigated by water from a small stream, and water samples were positive for Stx 2 by PCR. The identical VTEC O157 Stx 2 positive strain was isolated from the cases and in cattle at a farm upstream from the irrigation point. An active surveillance and reporting system was crucial and cooperation between all involved parties was essential for quickly identifying the cause of this outbreak. Handling of fresh greens from farm to table must be improved to minimize the risk of contamination.

Introduction

Infection with verotoxin-producing *Escherichia coli* (VTEC) has been recognized as a severe disease with bloody diarrhea and a cause of hemolytic uremic syndrome (HUS) and neurological symptoms since 1977 (Konowalchuk *et al*., 1977). The toxins that cause the disease were identified in 1982 (O'Brien *et al*., 1982; Karmali *et al*., 1983; O'Brien and LaVeck, 1983). There are two different cytotoxins that can be involved in the disease, Stx1 and Stx 2, and their subtypes or a combination of both. The microorganism is known all over the world and several large foodborne outbreaks have been linked to many different types of food. About 200 different serotypes are known and of these O157 has received most attention. VTEC O157 is also generally reported in foodborne outbreaks although other serotypes are more frequent in some parts of the world (Karch *et al*., 2005). Common sources for VTEC O157

[1]Department of Communicable Disease Control, Västra Götaland, Sweden.
[2]Department of Communicable Disease Control, Halland, Sweden.
[3]Environmental Administration Office, Göteborg, Sweden.
[4]Bacteriology Laboratory, Sahlgrens University Hospital, Göteborg, Sweden.
[5]Swedish Institute for Infectious Disease Control, Stockholm, Sweden.
[6]National Veterinary Institute, Uppsala, Sweden.
[7]Swedish Board of Agriculture, Jönköping, Sweden.
[8]Environmental Administration Office, Varberg, Sweden.

outbreaks are meat and meat products, unpasteurized milk and apple cider, bean sprouts, and lettuce (Bell *et al*., 1994; Hilborn *et al*., 1999, 2000; Breuer *et al*., 2001; Gillespie *et al*., 2003). A large outbreak was reported in the United States in 2006 where contaminated spinach was the cause of the outbreak (Anonymous, 2006).

The disease is a zoonosis and cattle are known to be an important reservoir for VTEC O157. The infectious dose is low, the incubation time is usually 3–4 days, and the routes of transmission are contaminated food or water, direct or indirect contact with infected cattle, and person-to-person contact (Su and Brandt, 1995). In Sweden, it has been mandatory to report cases of VTEC O157 in humans since 1996 and all serotypes since July 2004. About 200 cases of VTEC have been reported annually in the last several years in Sweden and most of them are of indigenous origin (Swedish Institute for Infectious Disease Control, http://www.smittskyddsinstitutet.se/). Most cases seem to be sporadic cases or smaller family outbreaks although a few community outbreaks have been identified (Ziese *et al*., 1996; Welinder-Olsson *et al*., 2004; Sartz *et al*., 2007).

It is notable that one subtype of O157:H7 and its variants have dominated in humans in Sweden since the late 1990s. This subtype was first isolated from a patient living on the east coast of Sweden about 15 years ago, and further cases were reported during 1995 and 1996. A more dramatic increase was recognized during 1997, especially on the west coast, and since then this area has had the highest incidence among humans. The first isolates from a farm of this subtype were in connection with a child who contracted the disease after drinking unpasteurized milk and had been playing in a barn. There have not been as many foodborne outbreaks detected in Sweden as in other countries. This may explain why one subtype and its variants prevail in human cases. This subtype is also common in cattle on the west coast of Sweden, where the prevalence is much higher than in other parts of the country. The aim of this paper is to describe the source of a large outbreak of VTEC O157 in the southwestern part of Sweden in 2005 and how it was determined.

The Start of the Outbreak

During the summer of 2005 more VTEC cases than expected were reported in the counties of Västra Götaland and Halland, located on the west coast of Sweden. However, the epidemiological investigations in early summer did not reveal any common source except for four persons attending the same party at the end of June. For the other cases recorded, no common source was found and the microbiological analyses showed several different subtypes. Normally, about 60–70 cases of VTEC per year are reported from this area, which has 1.8 million inhabitants.

In late August 2005, the number of reported cases of VTEC O157 increased substantially. The cases originated from different areas of the west coast of Sweden. In early September, the number of cases had risen to 36 in less than a week and continued to increase. An intensive investigation was undertaken by the Departments of Communicable Disease Control in Västra Götaland and Halland in cooperation with the local Environmental Health Protection Boards, the hospital laboratories, and the Swedish Institute for Infectious Disease Control (SMI) (Söderström *et al*., 2005).

Materials and Methods

Organization of the outbreak investigative team

As soon as the outbreak was discovered, an executive group was formed with representatives from the different departments and authorities involved in the epidemiological investigation. Telephone meetings were initially arranged every second day for the whole group and after each meeting information and statements from all involved departments were delivered to the public and media. People taking part in this investigation who were not in the executive group had the opportunity to join the phone conferences to get the latest information and take part in the discussions. Altogether more than 60 people took part in at least one of these telephone conferences The early message to the public from these meetings was to heat meat and rinse vegetables properly and to avoid any unpasteurized milk products because the source of the outbreak was still unknown.

Case finding and epidemiological studies

The patients were initially identified by the mandatory reporting system, but as soon as the outbreak was disclosed in the beginning of September 2005, active case finding was initiated. All health facilities in the area were informed about the outbreak (by fax and also by the websites of the Departments of Communicable Disease Control in Västra Götaland and Halland) and asked to carry out intensive surveillance and microbiological investigations of suspected cases. The cases were contacted by telephone by the Departments for Communicable Disease Control and interviewed with a standardized questionnaire about foods consumed and other relevant epidemiological information such as travel (domestic and abroad), contact with farms, outdoor swimming, etc., in the 7 days before onset of symptoms.

Cohort study. Several cases fell ill after having lunch at a restaurant on August 25. A cohort study with 55 people that visited the restaurant was performed. Fifty-one of them were interviewed with a standardized questionnaire about what food items from the menu they had consumed at the restaurant, if they had fallen ill, and type of symptoms. They were also asked to provide a stool sample for analysis. Stool samples were collected between September 2 and 5. Every item in the menu was cross-tabulated with being positive for VTEC as confirmed by the laboratory.

Case–control study. A matched case–control study was undertaken September 8 and 9 with 67 cases and 100 controls. Cases and controls were interviewed by telephone with a standardized questionnaire where one to three controls were interviewed for each case. The cases were asked about symptoms and what they had eaten the week before onset and controls what they had eaten the week before the interview. The questionnaire contained 73 items, including food products and activities such as visits to restaurants and at which shops they had bought their food etc. The controls were matched to each individual case by ± 3 years of age, sex, and residence in the same geographical area. Each item was analyzed separately using conditional logistic regression.

Distribution of suspected food items. The distribution of suspected food (from producers to shops and restaurants) revealed by the initial telephone interviews with the cases was investigated. As soon as lettuce was suspected, the Department of Environmental Health in Gothenburg investigated the distribution in the region from the farms to the different shops and restaurants by contacting the retailers and then trace-back investigations were performed.

Microbiological methods

Human. Most samples during the outbreak were initially analyzed and typed by the Bacteriological Laboratory at Sahlgrens University Hospital in Göteborg and later all isolates were analyzed by the SMI.

The specimens were analyzed using PCR to detect bacteria harboring the genes coding for Stx 1 or 2 (Svenungsson *et al.*, 2000; Welinder-Olsson *et al.*, 2000, 2002). PCR-positive specimens were recultured to isolate the bacteria harbouring the *Stx* genes. Epidemiological typing using pulsed-field gel electrophoresis (PFGE) analysis was mainly carried out as previously described (Swaminathan *et al.*, 2001) by using restriction enzyme *Xba*I and a CHEF mapper XA system (Bio-Rad Laboratories, Inc., Hercules, CA) except that the runtime was 26 hours at a voltage of 6 V/cm and the linearly ramped pulse time 12.5 to 40 seconds. Some samples were also subtyped by multiple-locus variable-number tandem-repeat analysis (MLVA) (Lindstedt *et al.*, 2004); this method permitted subtyping from patient specimens in which the VTEC bacteria were not identified by culture.

Water. The lettuce producers were contacted and samples from the environment at the farms as well as from the irrigation water were taken for analysis of VTEC. Water was also tested for indicator bacteria that would show the presence of fecal bacteria in the water. In the beginning of the environmental investigation, samples were taken from six different irrigation water sources from three different salad producers.

Altogether 30 water samples were taken from the stream that was used for irrigation of the lettuce from the suspected farm. In addition,

sludge and two sediment samples were also obtained at the intake of the irrigation plant.

Water samples were analyzed at SMI. Water samples were filtered and the filters pre-enriched in buffered peptone water, followed by immunomagnetic separation for *E. coli* O157 (Dynal Biotech ASA, Oslo, Norway) and cultivation on both Sorbitol Mac-Conkey agar (SMAC) and SMAC supplemented with cefixime and tellurite (CT-SMAC). In parallel, the pre-enrichment broth as well as the magnetic beads and the culture on the plates were screened by Stx and PCR (Svenungsson *et al.*, 2000). Attempts to isolate Stx-positive *E. coli* from PCR-positive samples were made.

Farms and animals. Fecal samples were collected from eight cattle farms situated upstream from where water was drawn to irrigate the lettuce involved in the outbreak. A total of 77 pooled samples from these farms, each consisting of 25 g cattle feces, were analyzed at the National Veterinary Institute (SVA) for VTEC O157. Analyses were performed according to Nordic Committee on Food Analysis (NMKL) method no. 164 (Anonymous, 2005) with a few modifications (immunomagnetic separation was performed only once, after 18–24 hours of pre-enrichment, and plating out was performed on only one agar medium, CT-SMAC). Isolated Stx-positive *E. coli* O157:H7 were analyzed at SMI using PFGE according to the PulseNet protocol.

Lettuce and other vegetables. Samples were taken from lettuce both at the farms and in different supermarkets as well as left-overs in the homes of some of the cases. Lettuce samples were analyzed at SVA for VTEC O157 according to NMKL method no. 164 (Anonymous, 2005).

Meteorological and harvest data. Records of temperature and rainfall data for the area were supplied by the Swedish Meteorological and Hydrological Institute (SMHI) and were collected at a meteorological station situated in Varberg, 13 km north of the implicated farm area.

Statistical methods

Fisher's exact test was then used to calculate odds ratios in the cohort study. Odds ratios in case–control study were calculated by conditional logistic regression (Hosmer and Lemeshow, 2000). A p-value < 0.05 was considered significant.

Results

Descriptive information

The geographic spread of the cases indicated a locally manufactured product. The initial interviews did not give any indications of meat, minced meat, or fermented sausage as the incriminated food item. None of the patients had consumed unpasteurized milk products and at least one of the cases was a vegetarian. Suspicion that some vegetable was the source of the outbreak was raised at an early stage. There were few children and many young women among the early interviewed cases. In the questionnaires, several persons reported having consumed lettuce, either at home or in different restaurants. Trace-back investigations of the lettuce consumed implicated a local lettuce producer. The lettuce producer withdrew his products from the market on September 8 and from September 9 no more lettuce was sold. After the lettuce was withdrawn from the market, there were only three more cases and all had bought lettuce before it was withdrawn (Fig. 1).

Patients

Altogether there were 135 cases that could be linked to this outbreak. Some of the cases had fallen ill as early as in the beginning of July (Fig. 1). The mean age was 35.8 (range 0–90) years and 64% were women (Fig. 2). Most of the patients had diarrhea with abdominal pain and blood in the stools. Seven cases were asymptomatic (sampled at trace-back). Eleven cases of HUS were identified, with a mean stay in hospital of 55 days. There were no deaths. Most of the cases were from the west coast of Sweden or had visited this area, but a few cases contracted the disease in other parts of the country (Fig. 3). The latter could be traced back to having consumed lettuce from the same producer.

Epidemiological studies

Cohort study. Twenty-one out of 51 persons included in the study had had diarrhea, stomach

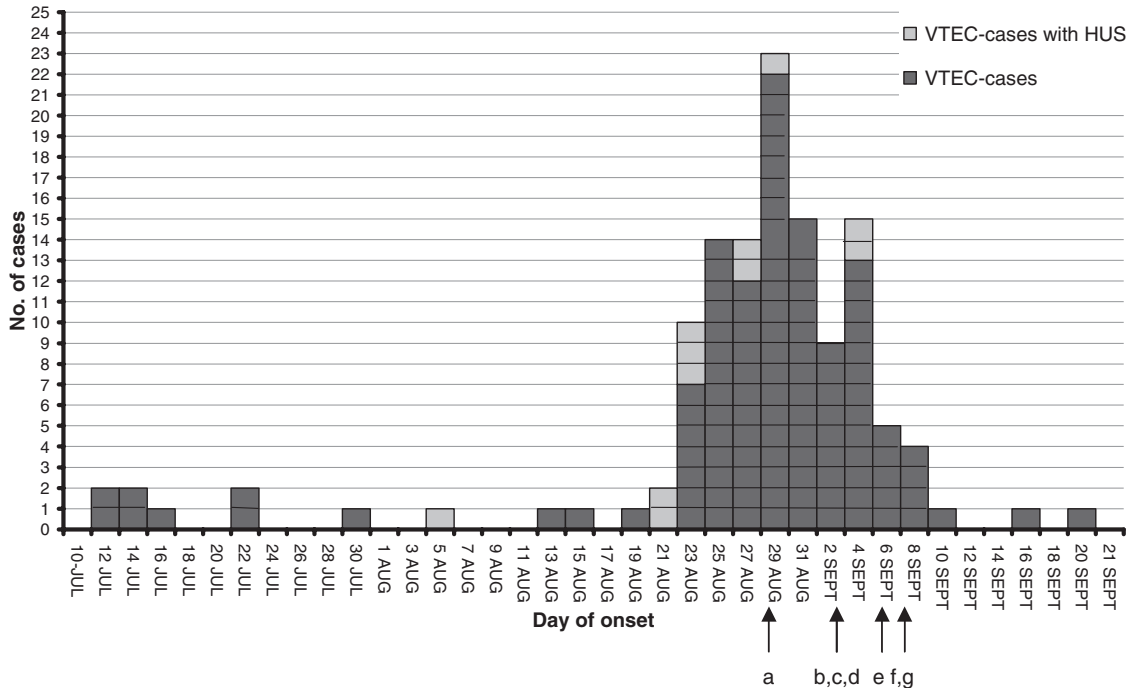

FIG. 1. Epidemic curve of a verotoxin-producing *Escherichia coli* (VTEC) outbreak in Sweden, 2005, for 126 out of 135 cases (for two cases the day of onset of illness is missing and seven cases were asymptomatic). Reporting date is in general 4–7 days later, sometimes longer. a, August 29: first case reported; b, September 2: cohort study; c, September 2 fax/info physician/clinic; d, September 2 press release; e, September 6 water samples; f, September 8 withdrawal of the product; g, September 8 case–control study.

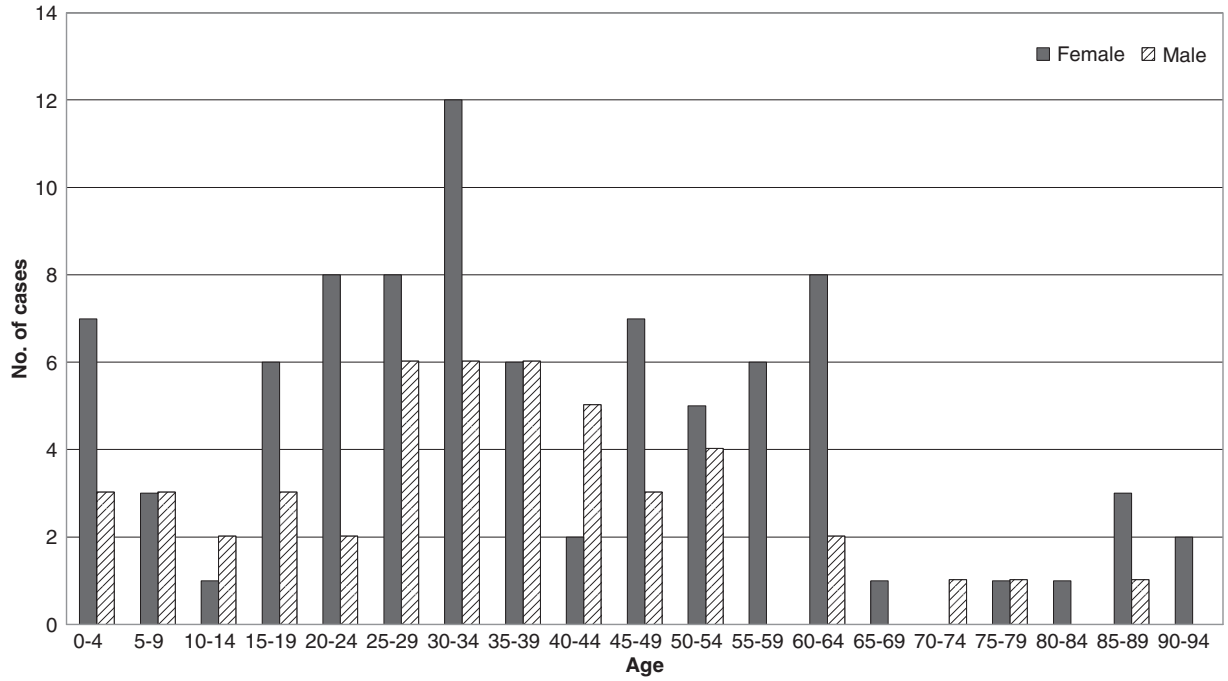

FIG. 2. Age distribution and sex of the cases in a verotoxin-producing *Escherichia coli* (VTEC) outbreak in Sweden, 2005.

FIG. 3. Distribution of the cases in the verotoxin-producing *Escherichia coli* (VTEC) outbreak in Sweden, 2005. Counties with cases: O, Västra Götaland; N, Halland; F, Jönköping; E, Östergötland; AB, Stockholm; M, Skåne; X, Gävleborg; Z, Jämtland; BD, Norrbotten. 1, The implicated lettuce producer; 2, water intake. The farm with identical VTEC strain as in the human cases was located upstream the water intake.

pain, nausea, and/or vomiting. Nine persons (20%) out of 45 tested were positive for VTEC. Many stool samples were taken rather late, when some of the cases had already recovered. Most of the persons had eaten the full buffet, which included bread, pasta salad, lasagne with seafood, green salad, and cheese. There was no association between different food items and the risk of disease as most of the visitors had eaten the same food items.

Case–control study. Three different food items were significantly associated with illness in the case–control study (Table 1), together with a visit to a restaurant or pizzeria. The strongest epidemiological link with VTEC infection was the consumption of iceberg lettuce, with an odds ratio of 13 (95% CI: 2.9–57.5). To obtain an estimate and confidence interval in this case, however, it became necessary to restratify, since no discordant strata were found (i.e., there was no strata in which a case had not eaten salad and at least one control had). The new matched strata were based on sex and birthyear categorized in 5-year intervals; in these new strata it was possible to have several cases and controls within each, and discordant strata were included.

The other significant food items, ice cream and alfalfa sprouts, however, had been eaten by very few people; for this reason they could not be considered to be plausible sources of infection. Most of the people who had eaten at a restaurant or pizzeria had also consumed iceberg lettuce.

Table 1. Odds Ratio (OR) with 95% Confidence Interval (CI) and *p*-Value for the 10 Items with Smallest *p*-Values in the Case–Control Study

Food item/place visit	*OR*	*95% CI lower*	*95% CI upper*	p-*value*
Iceberg lettuce[a]	13.0	2.94	57.50	< 0.001
Packaged ice cream	2.4	1.12	5.16	0.0185
Alfalfa sprouts	1.5	1.13	16.30	0.0205
Pizzeria	2.4	0.99	5.68	0.0467
Restaurant	2.0	0.99	4.15	0.0474
Leaf lettuce	1.9	0.98	3.81	0.0557
Herbal spices	2.0	0.92	4.24	0.0737
Unpackaged ice cream	2.1	0.88	4.96	0.0897
Tomato	3.4	0.67	17.30	0.111
Hard cheese	2.5	0.70	8.87	0.132

[a]Based on different matching (see *Results*).

Microbiological results

Human. Altogether 108 out of the 135 cases could be linked to this outbreak by an identical VTEC strain based on PFGE analysis (Fig. 4). A further eight patients were judged to be included in the outbreak for epidemiological reasons together with the results of MLVA. In 19 cases the samples were positive according to PCR but the Stx-producing bacteria could not be identified by culture. All these cases had an epidemiological link to the outbreak (family member or other close contact with a case or consumption of the same lettuce as a PFGE-confirmed case).

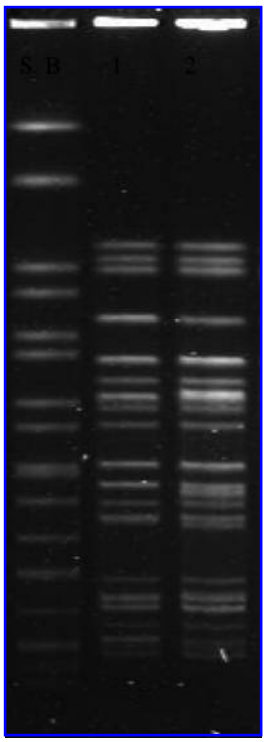

FIG. 4. Pulsed-field gel electrophoresis (PFGE) patterns of *E. coli* O157 strains. 1, Most common pattern among human isolates in Sweden; 2, pattern associated with the outbreak. S.B. is the size standard *Salmonella* Braenderup as recommended by PulseNet.

Water samples. Samples were taken from six different irrigation water sources from three different lettuce growers. Later on, when a specific salad grower was incriminated, water samples were taken from nine places in the stream that was used for irrigation of the lettuce on this farm and one from an inflow to the stream. They were sampled between September 6 and 22. Some of the samples were taken from different levels in the stream; altogether, 17 samples were taken. Further environmental samples were taken from the community sewage outlet (two), drainage well (two), sediment (two), and one sludge sample. Twelve of the water samples showed more than 24,000 coliforms/100 mL and *E. coli* between 150/100 mL and $>$24,000/100 mL. This means that those water samples were not potable and most were not even acceptable for outdoor swimming.

In six water samples from the stream used for irrigation on the farm (including the point of intake of irrigation water), Stx 2 was found with the PCR test. Additionally, Stx 1 was found in the sewage outlet water and in the inflow water to the stream. The VTEC bacteria could not be isolated by culture. All other water samples were negative for Stx1 and Stx 2.

Farms and animals. In the trace-back investigation, fecal samples from eight cattle farms

located upstream from where the water was taken for irrigation were analyzed. Altogether, 77 fecal samples were collected and 25 were positive for VTEC O157. These originated from four different farms. From one farm the PFGE pattern was exactly the same as among the human cases in this outbreak. In addition, two samples from drainage wells were positive for Stx 2 with PCR. One of the wells was situated in the area where all the cattle gather before entering the stable and the other well was in a field with grain earlier fertilized with manure from the farm. However, this particular farm was not right next to the stream from which the water for irrigation was taken, and all four samples of sludge from this farm were negative. Three more environmental samples from the farm where cattle had the same PFGE pattern as the ill people were also positive (two from walls and one from a strip of wood).

Lettuce and other vegetables. Twenty-nine samples were taken from the lettuce at the implicated farm, three samples from other producers, and some samples from different supermarkets in addition to samples from left-overs in the refrigerators of the cases. Altogether 44 samples of different types of lettuces and four samples of broccoli were investigated. VTEC O157 was not detected in any of the analyzed samples.

Weather and lettuce production. Data from the SMHI showed a dry period with little or no rain late in June and more rain later on in July and August (Fig. 5). Several batches of lettuce were produced during this period and the outbreak could not be traced back to a single batch.

Discussion

This is the largest outbreak of VTEC ever recorded in Sweden. Most of the cases were young

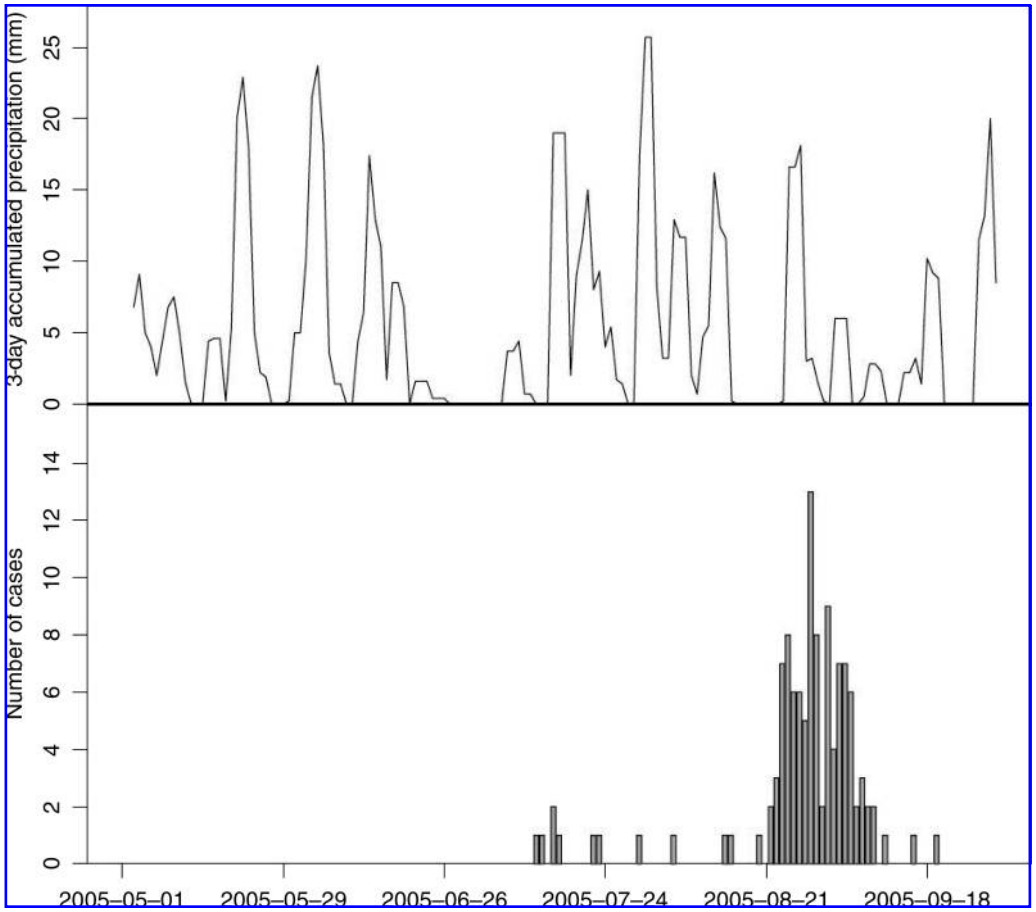

FIG. 5. Data on rainfall during and before the verotoxin-producing *Escherichia coli* (VTEC) outbreak in Sweden, 2005. Accumulated precipitation from 3 days interval (mm).

adults and more women than men were affected. This type of demographic pattern is common when the source of infection is any type of vegetable (Ackers *et al*., 1998; Hilborn *et al*., 1999). There were 11 cases of HUS (8%) but no fatal cases and the relatively few children and elderly people among the cases could probably explain these results. Internationally, 2–7% of the VTEC cases contract HUS but sometimes up to 30%, and the highest risk is among children under the age of 5 years and elderly persons (Akashi *et al*., 1994; Peacock *et al*., 2001). In earlier outbreaks in Sweden, between 0% (Welinder-Olsson *et al*., 2004) and 32% (Sartz *et al*., 2007) of the cases contracted HUS.

The early interviews and also the later case–control study showed that the most probable source of infection was lettuce. The risk of contracting VTEC after eating iceberg lettuce was 13 times higher compared with not having eaten it. This shows how important it is to conduct epidemiological studies in combination with other investigations, particularly if the microorganism could not be isolated from the suspected food item. In this outbreak, in spite of the large amount of lettuce produced and consumed, only 135 cases were reported. The farm consisted of a 28 hectare field with a production of 70,000 head of lettuce per hectare. The habit of rinsing lettuce before consumption might be efficient in reducing the number of microorganisms but cannot eliminate them (Beuchat and Ryu, 1997). From the interviews, it seemed more risky to consume lettuce at restaurants than at home. This could perhaps be explained by different methods of handling lettuce, which have been demonstrated earlier in foodborne outbreaks with lettuce (Kapperud *et al*., 1995). VTEC is not routinely looked for in all patients with diarrhea in Sweden and there could have been more cases than detected. Patients with mild symptoms might not have sought medical care.

Tracing the lettuce back to the producer was a huge task as the lettuce went through several different wholesalers. In some cases, the same batch of lettuce could be traced back to different restaurants and food shops where people had eaten or bought the lettuce and then fallen ill. Trace-back of these types of food that are handled by many wholesalers is crucial, can take a long time, and needs a lot of manpower, and if it is not done promptly, it can delay the outbreak investigation substantially. After the lettuce was withdrawn from the market, new cases more or less ceased. Only three persons contracted the disease after the withdrawal and all of them could be linked to the same lettuce producer. However, these persons had not received the information that the lettuce should not be consumed. The outbreak seemed to cease already before the withdrawal of the lettuce from the market. An explanation could be that the irrigation water was just temporary contaminated. Another explanation could be the intense media attention that already focused on lettuce as the suspected cause before the withdrawal.

The special subtype variant detected in this outbreak was first recognized in four people in the beginning of July, all attending a party in the outbreak area. Other variants of this subtype of VTEC have been isolated several times from cattle feces on the west coast. Similar but not identical strains have been detected in some smaller outbreaks in 1997 and 1999 in Sweden. During this outbreak investigation, VTEC O157 was isolated from several cattle farms in this area but only one had exactly the same pattern as the outbreak strain.

In spite of great efforts by the different laboratories involved, the bacteria could not be cultured from the lettuce. Earlier investigations of lettuce show that it is difficult to isolate the microorganism. The positive PCR samples from stream water and the intake pipe to the irrigation and the finding of the identical VTEC O157 strain in cattle upstream from the intake strengthen the hypothesis that the irrigation water was involved in the contamination of the lettuce in this outbreak. Isolation of the bacteria was not possible from the water, probably because of the high contamination with a lot of different enteric bacteria. However, the Stx 2 was detected by PCR from drainage wells at the farm with outlets in the stream and three environmental samples from the suspected farm were also positive. In addition, the case–control study and the cessation of the outbreak after withdrawal of the lettuce gives evidence of the source of this outbreak.

Irrigation of vegetables with water from local streams is common in Sweden. On this incriminated farm, some of the lettuce was irrigated

the day before and the day of harvest, which might have increased the risk of contamination. The producer cultivated a lot of lettuce during the whole season and the question is why this outbreak happened during the last part of August and not earlier. One explanation could be heavy rain that had caused the manure to be drained out to the stream, but there was a lot of rain during the whole summer (Fig. 5). In this particular area, where the lettuce was grown, it is known that about 23% of the cattle herds are carriers of VTEC O157 (Eriksson *et al*. 2005). This is a rural area with a lot of cattle farms. The samples of stream water showed that the water was highly polluted and not potable, and not even acceptable for outdoor swimming. A village sewage treatment plant as well as private drains into the stream and several farms with cattle not far from the stream increase the risk of fecal contamination, especially after heavy rain. The responsibility for the quality of the water used for vegetable production rests with the producer and at the time of the outbreak there were no specific recommendations.

According to the Swedish Food Act and EU legislation, growing vegetables are first regarded as a food product after harvesting and the only restriction is that the water should be suitable for the purpose. After this outbreak, a governmental commission was formed to produce a risk profile of VTEC, identify areas where knowledge is insufficient, and come up with measures that can be implemented to reduce the number of human VTEC cases and follow the whole chain from stable to table (Anonymous, 2007). Recommendations from the commission were that the irrigation water for vegetables should at least be approved as stated for outdoor swimming water.

Outbreaks of foodborne illnesses associated with consumption of fresh vegetables and fruits have become more common (Sivapalasingam *et al*., 2004; Rangel *et al*., 2005). These items can be contaminated in the field from manure or contaminated irrigation water, during processing or transport, or through contaminated storage. This is a challenge as the infectious dose is low and the consumer cannot eliminate the bacteria as can be done by heating meat and meat products. Once a product or food item that is intended to be consumed raw is contaminated it is difficult to prevent illness in the consumers. Handling of these fresh greens from farm to table must be improved to ensure that the risk of illness is minimized. The responsibility for this must be shared by all involved parties, from growers to distributors, retailers, and restaurants and also by the authorities.

This outbreak was detected in late August, and after about a week the most likely source was revealed and the lettuce was withdrawn from the market. There was very good cooperation between all involved departments, and the fast handling of the outbreak was the result of great efforts from all people involved.

Acknowledgment

We are very grateful to all the patients who answered the questionnaires about the food consumed before they fell ill.

References

Ackers ML, Mahon BE, Leahy E, Goode B, Damrow T, Hayes PS, Bibb WF, Rice DH, Barrett TJ, Hutwagner L, Griffin PM, and Slutsker L. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. J. Infect. Dis. 1998;**177:**1588–1593.

Akashi S, Joh K, Tsuji A, Ito H, Hoshi H, Hayakawa T, Ihara J, Abe T, Hatori M, Mori T, *et al*. A severe outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7 in Japan. Eur. J. Pediatr. 1994;**153:**650–655.

Anonymous. *Escherichia coli* O157. Detection in food and feeding stuff. Nordic Committee on Food Analysis, The Veterinary Institute, Oslo, Norway, 2005.

Anonymous. Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach—United States, September 2006. Morb. Mortal. Wkly. Rep. 2006;**55:**1045–1046.

Anonymous. Vertoxinbildande *E coli*, VTEC bakteriers smittvägar, förekomst samt risker för folkhälsan. Report from the National Food Administration, the Swedish Board of Agriculture, the National Veterinary Institute, the Swedish Institute for Infectious Disease Control, the National Board of Health and Welfare, and the Swedish Environmental Protection Agency, 2007. (In Swedish.)

Bell BP, Goldoft M, Griffin PM, Davis MA, Gordon DC, Tarr PI, Bartleson CA, Lewis JH, Barrett TJ, and Wells JG. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. JAMA 1994;**272:**1349–1353.

Beuchat LR and Ryu JH. Produce handling and processing practices. Emerg. Infect. Dis. 1997;**3:**459–465.

Breuer T, Benkel DH, Shapiro RL, Hall WN, Winnett MM, Linn MJ, Neimann J, Barrett TJ, Dietrich S, Downes FP,

Toney DM, Pearson JL, Rolka H, Slutsker L, and Griffin PM. A multistate outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. Emerg. Infect. Dis. 2001;**7:**977–982.

Eriksson E, Aspan A, Gunnarsson A, and Vagsholm I. Prevalence of verotoxin-producing *Escherichia coli* (VTEC) 0157 in Swedish dairy herds. Epidemiol. Infect. 2005;**133:**349–358.

Gillespie IA, Adak GK, O'Brien SJ, and Bolton FJ. Milk-borne general outbreaks of infectious intestinal disease, England and Wales, 1992–2000. Epidemiol. Infect. 2003;**130:**461–468.

Hilborn ED, Mermin JH, Mshar PA, Hadler JL, Voetsch A, Wojtkunski C, Swartz M, Mshar R, Lambert-Fair MA, Farrar JA, Glynn MK, and Slutsker L. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Arch. Intern. Med. 1999;**159:**1758–1764.

Hilborn ED, Mshar PA, Fiorentino TR, Dembek ZF, Barrett TJ, Howard RT, and Cartter ML. An outbreak of *Escherichia coli* O157:H7 infections and haemolytic uraemic syndrome associated with consumption of unpasteurized apple cider. Epidemiol. Infect. 2000;**124:**31–36.

Hosmer DW and Lemeshow S. 2000. *Applied Logistic Regression*. Wiley, New York, 2000.

Kapperud G, Rorvik LM, Hasseltvedt V, Hoiby EA, Iversen BG, Staveland K, Johnsen G, Leitao J, Herikstad H, Andersson Y, *et al*. Outbreak of *Shigella sonnei* infection traced to imported iceberg lettuce. J. Clin. Microbiol. 1995;**33:**609–614.

Karch H, Tarr PI, and Bielaszewska M. Enterohaemorrhagic *Escherichia coli* in human medicine. Int. J. Med. Microbiol. 2005;**295:**405–418.

Karmali MA, Petric M, Lim C, Fleming PC, and Steele BT. *Escherichia coli* cytotoxin, haemolytic-uraemic syndrome, and haemorrhagic colitis. Lancet 1983;**2:**1299–1300.

Konowalchuk J, Speirs JI, and Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect. Immun. 1977;**18:**775–779.

Lindstedt BA, Vardund T, and Kapperud G. Multiple-locus variable-number tandem-repeats analysis of *Escherichia coli* O157 using PCR multiplexing and multicolored capillary electrophoresis. J. Microbiol. Methods 2004;**58:**213–222.

O'Brien AD and LaVeck GD. Purification and characterization of a *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. Infect. Immun. 1983;**40:**675–683.

O'Brien AD, LaVeck GD, Thompson MR, and Formal SB. Production of *Shigella dysenteriae* type 1-like cytotoxin by *Escherichia coli*. J. Infect. Dis. 1982;**146:**763–769.

Peacock E, Jacob VW, and Fallone SM. *Escherichia coli* O157:H7: etiology, clinical features, complications, and treatment. Nephrol. Nurs. J. 2001;**28(5):**547–550, 553–545; quiz 556–547.

Rangel JM, Sparling PH, Crowe C, Griffin PM, and Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. Emerg. Infect. Dis. 2005;**11:**603–609.

Sartz L, De Jong B, Hjertqvist M, Plym-Forshell L, Alsterlund R, Lofdahl S, Osterman B, Stahl A, Eriksson E, Hansson HB, and Karpman D. An outbreak of *Escherichia coli* O157:H7 infection in southern Sweden associated with consumption of fermented sausage; aspects of sausage production that increase the risk of contamination. Epidemiol. Infect. 2008;**136:**370–380. (Epub 2007 Apr 20).

Sivapalasingam S, Friedman CR, Cohen L, and Tauxe RV. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J. Food Prot. 2004;**67:**2342–2353.

Söderström A, Lindberg A, and Andersson Y. EHEC O157 outbreak in Sweden from locally produced lettuce. Available at http://www.eurosurveillance.org/ViewArticle.aspx?ArticleID=2794. Eurosurveillance 2005; **10(9)**.

Su C and Brandt LJ. *Escherichia coli* infection in humans. Ann. Intern. Med. 1995;**123:**698–714.

Svenungsson B, Lagergren A, Ekwall E, Evengard B, Hedlund KO, Karnell A, Lofdahl S, Svensson L, and Weintraub A. Enteropathogens in adult patients with diarrhea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases. Clin. Infect. Dis. 2000;**30:**770–778.

Swaminathan B, Barrett TJ, Hunter SB, and Tauxe RV. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg. Infect. Dis. 2001;**7:**382–389.

Welinder-Olsson C, Badenfors M, Cheasty T, Kjellin E, and Kaijser B. Genetic profiling of enterohemorrhagic *Escherichia coli* strains in relation to clonality and clinical signs of infection. J. Clin. Microbiol. 2002;**40:**959–964.

Welinder-Olsson C, Kjellin E, Badenfors M, and Kaijser B. Improved microbiological techniques using the polymerase chain reaction and pulsed-field gel electrophoresis for diagnosis and follow-up of enterohaemorrhagic *Escherichia coli* infection. Eur. J. Clin. Microbiol. Infect. Dis. 2000;**19:**843–851.

Welinder-Olsson C, Stenqvist K, Badenfors M, Brandberg A, Floren K, Holm M, Holmberg L, Kjellin E, Marild S, Studahl A, and Kaijser B. EHEC outbreak among staff at a children's hospital—use of PCR for verocytotoxin detection and PFGE for epidemiological investigation. Epidemiol. Infect. 2004;**132:**43–49.

Ziese T, Anderson Y, De Jong B, Lofdahl S, and Ramberg M. Outbreak of *Escherichia coli* O157 in Sweden. Euro. Surveill. 1996;**1:**2–3.

Address reprint requests to:
Ann Söderström, M.D., Ph.D.
Smittskyddsenheten
Kaserntorget 11 B
411 18 Göteborg
Sweden

E-mail: ann.soderstrom@vgregion.se

This article has been cited by:

1. Matheus-Guimarães Cecilia, Gonçalves Evanilde Maria, Cabilio Guth Beatriz E.. 2014. Interactions of O157 and Non-O157 Shiga Toxin–Producing Escherichia coli (STEC) Recovered from Bovine Hide and Carcass with Human Cells and Abiotic Surfaces. *Foodborne Pathogens and Disease* **11**:3, 248-255. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Kevin Holvoet, Imca Sampers, Marleen Seynnaeve, Mieke Uyttendaele. 2014. Relationships among hygiene indicators and enteric pathogens in irrigation water, soil and lettuce and the impact of climatic conditions on contamination in the lettuce primary production. *International Journal of Food Microbiology* **171**, 21-31. [CrossRef]
3. Karin A. Nyberg, Björn Vinnerås, Ann Albihn. 2014. Managing Salmonella Typhimurium and Escherichia coli O157:H7 in soil with hydrated lime – An outdoor study in lysimeters and field plots. *Journal of Environmental Science and Health, Part B* **49**:1, 45-50. [CrossRef]
4. Mehboob Alam, Christine Ahlström, Stephen Burleigh, Crister Olsson, Siv Ahrné, Mohamed El-Mogy, Göran Molin, Paul Jensén, Malin Hultberg, Beatrix W. Alsanius. 2014. Prevalence of Escherichia coli O157:H7 on spinach and rocket as affected by inoculum and time to harvest. *Scientia Horticulturae* **165**, 235-241. [CrossRef]
5. John P. Brooks, Charles GerbaBioaerosol Contamination of Produce 107-121. [CrossRef]
6. Karin A Nyberg, Jakob R Ottoson, Björn Vinnerås, Ann Albihn. 2014. Fate and survival of Salmonella Typhimurium and Escherichia coli O157:H7 in repacked soil lysimeters after application of cattle slurry and human urine. *Journal of the Science of Food and Agriculture* n/a-n/a. [CrossRef]
7. Charles P. Gerba, Channah RockWater Quality 123-138. [CrossRef]
8. Karl R. MatthewsLeafy Vegetables 187-206. [CrossRef]
9. Sam Van Haute, Imca Sampers, Liesbeth Jacxsens, Mieke Uyttendaele. 2013. Selection Criteria for Water Disinfection Techniques in Agricultural Practices. *Critical Reviews in Food Science and Nutrition* 131126062226003. [CrossRef]
10. Jan Soon, Richard BainesManaging Risks in the Horticultural Sector 17-38. [CrossRef]
11. Maryam Azizkhani, Patricia Elizaquível, Gloria Sánchez, María Victoria Selma, Rosa Aznar. 2013. Comparative efficacy of Zataria multiflora Boiss., Origanum compactum and Eugenia caryophyllus essential oils against E. coli O157:H7, feline calicivirus and endogenous microbiota in commercial baby-leaf salads. *International Journal of Food Microbiology* **166**:2, 249-255. [CrossRef]
12. Inge Van der Linden, Bart Cottyn, Mieke Uyttendaele, Geertrui Vlaemynck, Marc Heyndrickx, Martine Maes. 2013. Survival of Enteric Pathogens During Butterhead Lettuce Growth: Crop Stage, Leaf Age, and Irrigation. *Foodborne Pathogens and Disease* **10**:6, 485-491. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental Material]
13. J.M. Soon, P. Seaman, R.N. Baines. 2013. Escherichia coli O104:H4 outbreak from sprouted seeds. *International Journal of Hygiene and Environmental Health* **216**:3, 346-354. [CrossRef]
14. Hon-Ming Lam, Justin Remais, Ming-Chiu Fung, Liqing Xu, Samuel Sai-Ming Sun. 2013. Food supply and food safety issues in China. *The Lancet* **381**:9882, 2044-2053. [CrossRef]
15. Cheng Liu, Nynke Hofstra, Eelco Franz. 2013. Impacts of climate change on the microbial safety of pre-harvest leafy green vegetables as indicated by Escherichia coli O157 and Salmonella spp. *International Journal of Food Microbiology* **163**:2-3, 119-128. [CrossRef]
16. Adrian Sbodio, Sharyn Maeda, Gabriela Lopez-Velasco, Trevor V. Suslow. 2013. Modified Moore swab optimization and validation in capturing E. coli O157:H7 and Salmonella enterica in large volume field samples of irrigation water. *Food Research International* **51**:2, 654-662. [CrossRef]
17. Guiomar D. Posada-Izquierdo, Fernando Pérez-Rodríguez, Francisco López-Gálvez, Ana Allende, María V. Selma, María I. Gil, Gonzalo Zurera. 2013. Modelling growth of Escherichia coli O157:H7 in fresh-cut lettuce submitted to commercial process conditions: Chlorine washing and modified atmosphere packaging. *Food Microbiology* **33**:2, 131-138. [CrossRef]
18. Inge Van der Linden, Bart Cottyn, Mieke Uyttendaele, Geertrui Vlaemynck, Martine Maes, Marc Heyndrickx. 2013. Long-term survival of Escherichia coli O157:H7 and Salmonella enterica on butterhead lettuce seeds, and their subsequent survival and growth on the seedlings. *International Journal of Food Microbiology* **161**:3, 214-219. [CrossRef]
19. J. Elder, K. NightingaleTracking of pathogens via virulence factors: Shiga toxin-producing Escherichia coli in cattle and potential risks for human disease 227-259. [CrossRef]
20. S. Baugh, A. S. Ekanayaka, L. J. V. Piddock, M. A. Webber. 2012. Loss of or inhibition of all multidrug resistance efflux pumps of Salmonella enterica serovar Typhimurium results in impaired ability to form a biofilm. *Journal of Antimicrobial Chemotherapy* **67**:10, 2409-2417. [CrossRef]

21. Emily MacDonald, Berit Tafjord Heier, Karin Nygård, Torunn Stalheim, Kofitsyo S. Cudjoe, Taran Skjerdal, Astrid Louise Wester, Bjørn-Arne Lindstedt, Trine-Lise Stavnes, Line Vold. 2012. Yersinia enterocolitica Outbreak Associated with Ready-to-Eat Salad Mix, Norway, 2011. *Emerging Infectious Diseases* **18**:9, 1496-1499. [CrossRef]
22. Jan-Mei Soon. 2012. Food safety perceptions and practices of selected UK fresh produce farms. *Quality Assurance and Safety of Crops & Foods* n/a-n/a. [CrossRef]
23. J.M. Monaghan, M.L. Hutchison. 2012. Distribution and decline of human pathogenic bacteria in soil after application in irrigation water and the potential for soil-splash-mediated dispersal onto fresh produce. *Journal of Applied Microbiology* no-no. [CrossRef]
24. D.T. Ingram, M.T. Callahan, S. Ferguson, D.G. Hoover, D.R. Shelton, P.D. Millner, M.J. Camp, J.R. Patel, K.E. Kniel, M. Sharma. 2012. Use of zero-valent iron biosand filters to reduce Escherichia coli O157:H12 in irrigation water applied to spinach plants in a field setting. *Journal of Applied Microbiology* no-no. [CrossRef]
25. Hassan Vally, Gillian Hall, Amalie Dyda, Jane Raupach, Katrina Knope, Barry Combs, Patricia Desmarchelier. 2012. Epidemiology of Shiga toxin producing Escherichia coli in Australia, 2000-2010. *BMC Public Health* **12**:1, 63. [CrossRef]
26. P. Elizaquível, G. Sánchez, M.V. Selma, R. Aznar. 2011. Application of propidium monoazide-qPCR to evaluate the ultrasonic inactivation of Escherichia coli O157:H7 in fresh-cut vegetable wash water. *Food Microbiology* . [CrossRef]
27. Christina Frank, Dirk Werber, Jakob P. Cramer, Mona Askar, Mirko Faber, Matthias an der Heiden, Helen Bernard, Angelika Fruth, Rita Prager, Anke Spode, Maria Wadl, Alexander Zoufaly, Sabine Jordan, Markus J. Kemper, Per Follin, Luise Müller, Lisa A. King, Bettina Rosner, Udo Buchholz, Klaus Stark, Gérard Krause. 2011. Epidemic Profile of Shiga-Toxin–Producing Escherichia coli O104:H4 Outbreak in Germany. *New England Journal of Medicine* 111026144204001. [CrossRef]
28. Anita Forslund, Finn Plauborg, Mathias Neumann Andersen, Bo Markussen, Anders Dalsgaard. 2011. Leaching of human pathogens in repacked soil lysimeters and contamination of potato tubers under subsurface drip irrigation in Denmark. *Water Research* . [CrossRef]
29. Lindsey A. Keskinen, Bassam A. Annous. 2011. Efficacy of adding detergents to sanitizer solutions for inactivation of Escherichia coli O157:H7 on Romaine lettuce. *International Journal of Food Microbiology* **147**:3, 157-161. [CrossRef]
30. Yakov Pachepsky, Daniel R. Shelton, Jean E.T. McLain, Jitendra Patel, Robert E. MandrellIrrigation Waters as a Source of Pathogenic Microorganisms in Produce **113**, 73-138. [CrossRef]
31. Stelios Viazis, Francisco Diez-GonzalezEnterohemorrhagic Escherichia coli **111**, 1-50. [CrossRef]
32. Marjorie Bardiau, Sabrina Labrozzo, Jacques G Mainil. 2011. Study of polymorphisms in tir, eae and tccP2 genes in enterohaemorrhagic and enteropathogenic Escherichia coli of serogroup O26. *BMC Microbiology* **11**:1, 124. [CrossRef]
33. Yakov Pachepsky, Daniel R. Shelton, Jean E.T. McLain, Jitendra Patel, Robert E. MandrellIrrigation Waters as a Source of Pathogenic Microorganisms in Produce: A Review **113**, 75-141. [CrossRef]
34. R. E. MandrellTracing pathogens in fruit and vegetable production chains 548-595. [CrossRef]
35. Jie Wei, Kalmia E. Kniel. 2010. Pre-harvest Viral Contamination of Crops Originating from Fecal Matter. *Food and Environmental Virology* **2**:4, 195-206. [CrossRef]
36. Karin A. Nyberg, Björn Vinnerås, Jakob R. Ottoson, Pär Aronsson, Ann Albihn. 2010. Inactivation of Escherichia coli O157:H7 and Salmonella Typhimurium in manure-amended soils studied in outdoor lysimeters. *Applied Soil Ecology* **46**:3, 398-404. [CrossRef]
37. Cedric N. Berger, Samir V. Sodha, Robert K. Shaw, Patricia M. Griffin, David Pink, Paul Hand, Gad Frankel. 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology* **12**:9, 2385-2397. [CrossRef]
38. Inger Olesen, Lene Jespersen. 2010. Relative gene transcription and pathogenicity of enterohemorrhagic Escherichia coli after long-term adaptation to acid and salt stress. *International Journal of Food Microbiology* **141**:3, 248-253. [CrossRef]
39. Lindsey A. Keskinen, Angela Burke, Bassam A. Annous. 2009. Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate Escherichia coli O157:H7 from lettuce leaves#. *International Journal of Food Microbiology* **132**:2-3, 134-140. [CrossRef]
40. Karl R. MatthewsLeafy Vegetables 165-187. [CrossRef]
41. Charles P. Gerba, Christopher Y. ChoiWater Quality 105-118. [CrossRef]