

## EHEC outbreak among staff at a children's hospital – use of PCR for verocytotoxin detection and PFGE for epidemiological investigation

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### SUMMARY

This is the first report of a major foodborne outbreak of enterohaemorrhagic *Escherichia coli* (EHEC) in Sweden. It occurred among the nursing staff at a children's hospital with approximately 1600 employees. Contaminated lettuce was the most likely source of infection. Nine persons were culture-positive for *Escherichia coli* (*E. coli*) O157 and verocytotoxin-positive by PCR and a further two were verocytotoxin-positive by PCR only. All 11 EHEC-positive individuals had attended a party for approximately 250 staff members, which was held at the hospital. In a questionnaire 37 persons stated that they had symptoms consistent with EHEC infection during the weeks after the party. There was no evidence of secondary transmission from staff to patients. The value of PCR as a sensitive and fast method for diagnosis is discussed in this paper. Pulsed-field gel electrophoresis (PFGE) was used to ascertain that staff members were infected by the same clone, and that two patients with *E. coli* O157 infection were not.

### INTRODUCTION

Infections caused by enterohaemorrhagic *Escherichia coli* (EHEC) may, especially among children, lead to severe enterocolitis with complications such as microangiopathic haemolytic anaemia, thrombocytopenia and haemolytic uraemic syndrome (HUS) [1–3]. EHEC is usually detected by culture as sorbitol-negative (grey) colonies, in contrast to sorbitol-positive colonies that are red on MacConkey agar plates. The sorbitol negative (grey) colonies are tested for the presence of EHEC O157 by agglutination.

The most important virulence genes are the genes coding for the verocytotoxins (VT) 1 and 2. Since 1997, the Bacteriological Laboratory in Göteborg routinely screens, together with culture, for VT1- and/or VT2-harboured bacteria by polymerase chain reaction (PCR) among specimens from patients with bloody diarrhoea and all children younger than 11 years.

Cattle are the principal reservoir of EHEC and the majority of large outbreaks have been foodborne [4]. Meat, fruit and vegetable products, which presumably have come into contact with domestic animal manure and raw, or inadequately pasteurized, dairy products are important vehicles of infection [5]. Person-to-person transmission can be a significant

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means of dissemination in institutional settings, especially day-care centres and nursing homes [6].

Infections due to EHEC were rarely diagnosed in Sweden before 1994. Only 1–3 cases of EHEC were reported annually to the Swedish Institute for Infectious Disease Control [7]. After a considerable increase of cases during 1995, *E. coli* O157 was made notifiable in Sweden from January 1996. From July 1995 until the end of February 1996, a total of 110 cases of EHEC (*E. coli* O157), complicated by HUS in 26% of cases, were reported throughout the country. A case-control study was performed, but the source of the outbreak could not be identified [8]. Four subtypes of *E. coli* O157 were identified and it was considered that several sources of infection were involved [7]. During the summer and autumn of 1997, 27 cases of *E. coli* O157 were diagnosed at the Bacteriology Laboratory in Göteborg. Transmission was associated with animal contact, swimming in the same lake and secondary transmission. Altogether, seven PFGE patterns were identified [9]. Over the years, limited outbreaks due to infected food or non-pasteurized milk have occurred.

This is the first report of a foodborne outbreak of *E. coli* O157 in Sweden. It occurred in 1999 among the staff at a children's hospital. Herein, we discuss the difficulties that may arise when investigating an outbreak that takes place in such a setting. This outbreak also illustrates how the use of molecular biology techniques such as PCR for diagnosis of the virulence genes VT1 and VT2 coding for the two verocytotoxins in EHEC, and PFGE for epidemiological typing can be crucial for the analysis and control of an outbreak.

## MATERIALS AND METHODS

### Initial events and investigation

On 7 and 8 September 1999, two nurses from the children's hospital in Göteborg were admitted to the Clinic of Infectious Diseases due to bloody diarrhoea lasting 3 and 4 days. On 8 and 10 September respectively, their stool specimens were positive for VT2 as analysed by PCR, indicating EHEC infection. On 13 September both patients were diagnosed with *E. coli* O157 infection by culture. The two nurses were interviewed regarding recent food intake and activities, and it was soon apparent that both had attended a party for 250 members of staff at the children's hospital on 31 August. The Hospital Infection

Control Unit judged it likely that the nurses had been infected from a common source, probably contaminated food served from a buffet at the party. Instructions were given to sample patients and staff with diarrhoea at the two wards where the EHEC-positive nurses had been working before falling ill. Relatives of patients that had been discharged were contacted. Persons with diarrhoea were excluded from work.

### Further investigation of staff and patients

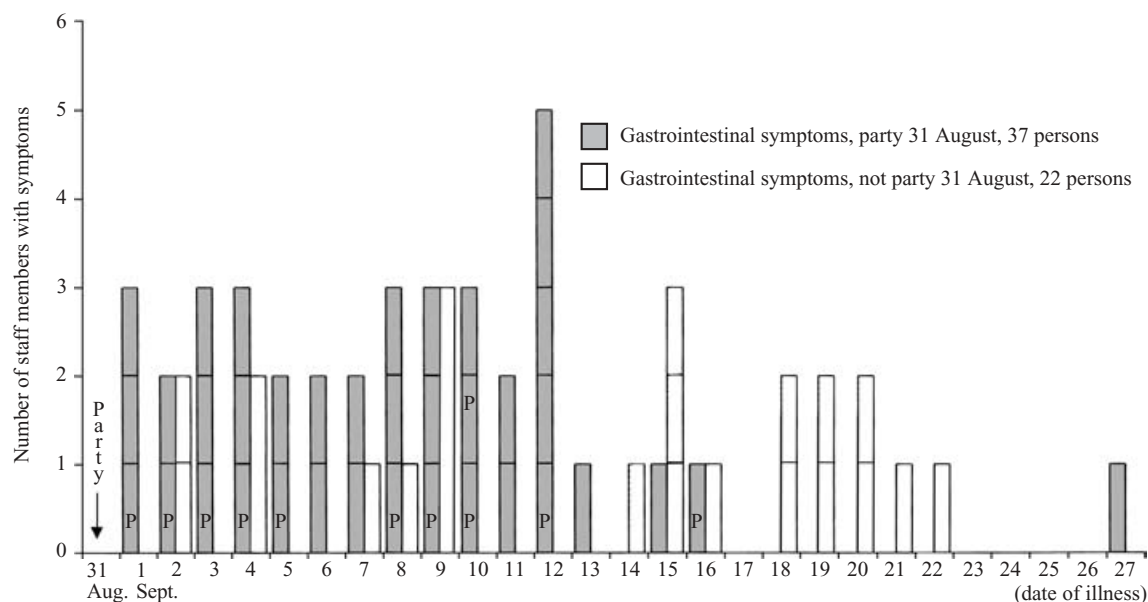
The Department of Communicable Disease Control was informed about the first cases on 10 September. After this date, the outbreak was managed in cooperation between this department, the Environmental Health Protection Agency, the hospital management and the Hospital Infection Control Unit. A formal strategic group was, however, not established.

On 15 September, instructions were sent to all wards that personnel with diarrhoea should provide stool specimens. The information as to whether or not it was possible to remain at work was inconsistent between different communicators, therefore staff with mild symptoms working at wards with older children might have chosen to stay at work. In addition, there was no clear consensus on the possibility of infection being transmitted by EHEC-positive personnel lacking symptoms. The Hospital Infection Control Unit stated that it was highly unlikely that nursing staff without diarrhoea would transmit the infection at the hospital. On the other hand, EHEC-positive individuals were not permitted to return to work until one stool specimen was negative.

### Party attendees and sampled individuals

The children's hospital had in the region of 1600 employees. Approximately 250 employees attended the party. Stool specimens, taken as rectal swabs, from 59 individuals were analysed by culture and PCR (see Microbiological methods). Stool specimens were sent to the laboratory at Sahlgrenska University Hospital, Göteborg, from persons with symptoms consistent with EHEC infection. Staff members were, however, not always sampled on the same day as the first symptoms. Thirty-seven of the 59 staff members had attended the party and 22 had not (Fig. 1).

Furthermore, all patients at the children's hospital with symptoms suggesting EHEC infection were routinely sampled.



**Fig. 1.** Date for onset of gastrointestinal symptoms for 59 of the staff members sampled. Thirty-seven of the 59 staff members had attended a party on 31 August. P, indicates EHEC diagnosed by culture and/or PCR.

## MICROBIOLOGICAL METHODS

### Culture

Ordinary sorbitol-containing, MacConkey agar plates (SMAC) were used for the culture of EHEC. The specimens were also cultured regarding *Salmonella*, *Shigella*, *Campylobacter* and *Yersinia* using standard procedures.

### PCR

PCR was performed as previously described [9, 10]. Briefly, bacterial growth from the primary agar plate culture of the human stool specimen, or from growth of the carrot and lettuce suspensions, was suspended in 4 ml of water to McFarland 4 and heated to 100 °C for 15 min. A 5 µl aliquot was used for each PCR. A multiplex PCR was used with primers detecting both VT1 and VT2 gene sequences. The PCR was performed in a total volume of 50 µl containing 200 µM of each dNTP, 2.5 mM MgCl<sub>2</sub>, 10 pmol of each primer VT1/ (5'-GAA GAG TCC GTG GGA TTA CG-3'), VT1r (5'-AGC GAT GCA GCT ATT AAT AA-3'), VT2/ (5'-ACC GTT TTT CAG ATT TT(G/A) CAC ATA-3') and VT2r (5'-TAC ACA GGA GCA GTT TCA GAC AGT-3'), 5 µl of 10 × concentrated polymerase synthesis buffer and 1.25 U Gold Taq polymerase (Applied Biosystems, Stockholm, Sweden). The PCR was performed in a Gene Amp PCR system 9600 (Applied Biosystems). The thermocycling started

with 10 min incubation at 94 °C followed by 20 s at 94 °C, 45 s at 55 °C and 10 s at 72 °C for 35 cycles.

### Pulsed-field gel electrophoresis (PFGE)

We used PFGE to establish clonal relation and diversity among the strains. Sample preparation was performed according to the method described by Gautom [11]. Restriction enzyme digestion was performed as described earlier [10]. Briefly, DNA was digested with 20 U of the enzyme *Xba*I following electrophoresis performed with the Gene path system (Bio-Rad Laboratories, Sundbyberg, Sweden). The supplier recommended the program used: no. 22 (Eco 157), with initial switch time of 2.2 s, final switch time of 54.2 s, run time of 22 h, angle of 120 °C, gradient of 6.6 V/cm, temperature of 14 °C and linear ramping factor. The PFGE types were interpreted according to Tenover et al. [12]. Isolates with no or 2–3 fragment differences compared to the outbreak pattern were considered related or probably related to the outbreak pattern. A total of 4–6 fragment differences were considered as possibly related, and 7 or more differences were considered not related.

### Serogrouping

The *E. coli* O157 test kit Oxoid Ltd (Basingstoke, UK) was used. Culture Collection, University of Göteborg (CCUG) strains, CCUG nos. 17620 and 29188 were used as negative and positive controls.

### Epidemiological investigation

EHEC-positive individuals were interviewed about symptoms, possible exposure other than the buffet and in detail about food intake at the buffet.

In order to exclude further cases and to analyse the extent of the outbreak, the Department of Communicable Disease Control distributed a questionnaire to all wards on 22 September. Personnel with symptoms of EHEC infection, defined as upset stomach, abdominal pain or diarrhoea after 31 August were asked to give the date of onset of their symptoms. They were also instructed to state whether they had been at work or on sick leave, and whether they had attended the party on 31 August.

### Environmental investigation

The buffet consisted of peanuts, prawns in warm sauce, grated carrots, chicken and lettuce served with mango juice and wine. The lettuce was imported from central Europe. The food was prepared by six of the regular cooks at the hospital. No food remained to be sampled, however, carrots and lettuce from the wholesalers who had supplied the vegetables for the party were analysed. A suspension of water that had been used to industrially wash the carrots was used for PCR and culture. Furthermore, a mixture of a quarter from each of three heads of lettuce and 50 ml of distilled water was homogenized in a Stomacher 400 [13] and analysed for verocytotoxin-producing bacteria with PCR and cultured for detection of EHEC, *Salmonella*, *Shigella*, *Campylobacter* and *Yersinia*.

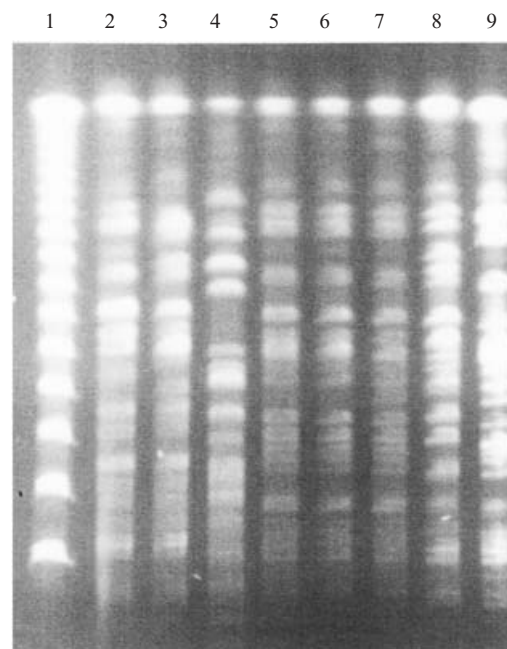
All six kitchen staff were sampled for EHEC.

## RESULTS

### Microbiological results

Fifty-nine members of the staff with symptoms were sampled. Eleven stool specimens were positive for VT2 with PCR, indicating EHEC infection (Fig. 1). No one was positive for VT1. Only staff members who attended the party were EHEC positive. In 9 of the 11 specimens, individual colonies of verocytotoxin-producing *E. coli* were isolated and these all agglutinated positive for serogroup O157.

In nine of the EHEC-positive individuals, the incubation time was between 8 h and 10 days. One person, who also was EHEC-positive by both PCR and culture, reported first symptoms 16 days after the



**Fig. 2.** PFGE band patterns. Lanes 5–7 are three of the outbreak strains isolated from the staff. Lanes 8, 9 are strains isolated from two patients who appeared not to be included in the outbreak but were treated for EHEC at the hospital at the time for the outbreak. Lanes 2–4 are EHEC strains of serogroup O157 isolated earlier in 1997 and 1998, epidemiologically unrelated to the outbreak strain as well as the patients strain. Lane 1 is a lambda ladder marker.

party. The strains from 8 of the 9 staff members had PFGE patterns identical to each other (Fig. 2). In one case, a two-band difference in the PFGE pattern was identified compared to the index case (Fig. 2, lane 7).

Altogether, 37 stool specimens were also taken from in-patients during September. Out of these, two children who were admitted to the hospital due to diarrhoea and HUS respectively during the first week of September, were diagnosed with *E. coli* O157 infection. Both had VT2-positive and VT1-negative strains belonging to serogroup O157. The PFGE profile of their strains differed from each other, as well as from the profile of the infected staff (Fig. 2). The strain in lane 8 differed from the outbreak strain with nine bands and the strain in lane 9 differed by five bands.

### Results of epidemiological investigation

According to the interviews of the 11 EHEC-positive individuals, the party on 31 August was the only possible common event of exposure.

Thirty-seven members of staff who had attended the party stated in the questionnaire, which was sent out on 22 September, that they had symptoms

indicating possible gastrointestinal infection after 31 August (Fig. 1). Twenty-two stated that they had symptoms without attending the party.

Forty-six of the symptomatic nursing staff remained at work, 6 were excluded from the working place and 11 were on sick leave.

### Results of environmental investigation

Culture from the suspension of washed carrots showed moderate growth dominated by *Bacillus subtilis* and sparse growth of yeast fungus and mould. PCR was negative for verocytotoxin-producing bacteria. Analysis of the suspension of lettuce showed massive growth of a mixture of Gram-negative bacteria ( $120 \times 10^6$ /quarter of head of lettuce) of the family Enterobacteriaceae, highly resistant to many antibiotics. Verocytotoxin-producing bacteria were not identified by PCR in the lettuce suspension.

The six members of the kitchen staff were negative for EHEC by PCR and culture.

## DISCUSSION

This is a report of the first major foodborne outbreak of EHEC infection in Sweden. It occurred among the staff at a children's hospital in late summer 1999. Approximately 250 out of 1600 employees attended a party, which was held at the hospital. Approximately 1 week later, two nurses were admitted to the Clinic of Infectious Diseases with bloody diarrhoea, both being infected with VT2-producing *E. coli* O157. The investigation revealed altogether 11 staff members with EHEC infection. All staff members infected with EHEC had attended the party. As is evident from Figure 1, there were other simultaneous gastrointestinal infections among staff members, such as Calicivirus, etc. There was no evidence of secondary transmission to the patients. Contaminated lettuce, which was served at the party, was the most likely source of infection.

PFGE is a useful method for establishing clonal relatedness and diversity in a probable epidemic outbreak [14, 15]. In this outbreak, 8 of 9 culture-positive staff members had isolates with an identical PFGE pattern. In one case, the strain (Fig. 2, lane 7) differed by two fragments from the others. It is probable that this person was infected from the same source as the other party members, since she was sampled at a later stage, 16 days after the party, increasing the possibility for a mutation. The PFGE patterns of the two patients differed by 5 and 9 bands respectively to

the strains of the nursing staff, and by 7 bands from each other. According to the criteria of Tenover et al. [12] it might be possible that the strain that differed by 5 bands from the outbreak strain could still be related to the outbreak. It is, however, highly unlikely that this patient was infected at the hospital since he was admitted with bloody diarrhoea and had no previous connection with the hospital.

All EHEC-positive individuals had attended the same party. A buffet with a limited number of dishes was served. It was judged unlikely that peanuts, prawns, wine or heat-treated mango juice would be contaminated by EHEC. Chicken was also served but it is not known to be a reservoir of EHEC. In addition, the internal controls from the food supplier were satisfactory. Grated carrots and lettuce remained as the potential source of the outbreak. A suspension from industrial carrot wash from the wholesaler, who supplied the carrots for the party, was analysed and found to be EHEC-negative by PCR and culture. Only sparse to moderate growth of normal environmental flora, such as *Bacillus subtilis*, yeast fungus and mould was detected. The fact that approximately 1000 members of staff had been served at lunchtime from the same delivery of carrots in the hospital canteen without complications also made it less likely that contaminated carrots could have caused the outbreak. Three heads of lettuce from the wholesaler, who supplied the lettuce served at the party, were analysed by culture and PCR. There was a massive growth of a mixture of Gram-negative bacteria of the family Enterobacteriaceae including *E. coli*, with an antibiotic-resistant pattern far more resistant than that usually seen in Sweden. These types of bacteria are usually seen in manure and can be seen as an indicator of contamination of EHEC [16]. Lettuce is grown with continuous fertilizing until harvest and lettuce also has to be irrigated. Among farms with intensive livestock production, the possibility increases for contamination of the lettuce during the fertilization process or by the irrigation water. Within the last decade, 17 foodborne outbreaks have been linked to contaminated lettuce or salad [17]. We are aware of two other foodborne outbreaks of EHEC O157 in acute care hospitals. In one outbreak in Canada in 1995, contaminated iceberg lettuce was the source of infection and 8 patients and 10 members of staff were affected [18]. In another outbreak in Scotland, 16 in-patients and 11 members of staff were infected and found to be stool culture-positive for EHEC O157 after eating cream cakes [19]. Food

provided within the hospital, other than at the party, was free from suspicion as a source of the infection as no patients or other staff members were infected.

The management of the outbreak at the children's hospital in Göteborg in September 1999 was complicated by the fact that the roles and responsibilities of the management, the Hospital Infection Control Unit and the Department of Communicable Disease Control were not fully clarified. Several telephone meetings were held, but a formal investigation group was not formed. Some of the leading consultants did not judge EHEC infection among the staff to be a high-risk situation, and thus did not see the need for a full investigation. This attitude may be due to the fact that experience of EHEC infections at the time was limited in Sweden. A complicating factor was that staff numbers were low, which meant that the nurses knew that their presence at work was necessary. Staff members may also have chosen to remain at work due to reduced payment during sick leave.

In retrospect, it is obvious that an authorized leader should have been appointed, roles and responsibilities defined and resources allocated in order to handle the complex situation caused by the outbreak. The 11 staff members who were identified with EHEC infection are likely to represent a minimal number of true cases. A further 30 persons having attended the party were sampled, and it cannot be ruled out that some of them had been infected by EHEC since many of them were sampled 2–3 weeks after the event. The epidemiological investigation was limited and delayed. The attack rate was not examined and a case-control study was not performed. A questionnaire was distributed to all wards 3 weeks after the party in order to rule out further cases. It showed that 59 persons had had symptoms consistent with EHEC infection at some time during the weeks following the party, and that 37 of these had attended the party on 31 August. Only 17 of the 59 symptomatic staff members were excluded from work or on sick leave.

There was no sign of transmission from staff to patients. This indicates that hygiene procedures were satisfactory. Secondary transmission can, however, not be excluded since one of the EHEC-positive staff members reported an onset of symptoms 16 days after the party (Fig. 2, lane 7). Long incubation periods of up to 14 days have, however, been reported in connection with EHEC infections [20, 21]. This outbreak shows the importance of hygiene procedures, the majority of cases having occurred before the full investigation started. It is important that nursing staff with acute

diarrhoea are sampled and removed from direct patient care. In addition, considering the low infectious dose in EHEC infections, nursing staff who are asymptomatic carriers should be transferred to administrative positions. Secondary transmission is most often seen among outbreaks of EHEC at nursing homes and psycho-geriatric wards [22, 23]. We are aware of only three reports from acute care hospitals [24–26].

The investigations of outbreaks are often complex involving epidemiological, as well as microbiological, methods. In addition, cooperation between different authorities and contacts with mass media are mandatory. One or two cases of diarrhoea among the staff at a big hospital may be overlooked if the number of cases at each individual ward is limited. This report demonstrates the importance of PCR and PFGE for the detection and analysis of an outbreak. The PCR result is usually ready after 1 day and is also a more sensitive method than culture [10, 27, 28]. The light colonies of sorbitol-negative *E. coli* O157 bacteria are often difficult to detect by culture, and in addition far from all EHEC bacteria are sorbitol-negative. If culture is preceded by PCR and the sample is verocytotoxin-positive, colonies of EHEC are more likely to be detected. Indeed, without the use of PCR, this outbreak would probably not have been identified. We routinely screen, all specimens from patients under 11 years of age with diarrhoea and specimens from older patients with sorbitol-negative colonies on sorbitol-MacConkey agar and/or reported diagnosis of severe or bloody diarrhoea, for verocytotoxin-positive *E. coli* by PCR. We believe this is an important step in diminishing the consequences of both outbreak situations and even single cases of EHEC as patients will get adequate treatment early. Finally, fingerprinting by PFGE proved that EHEC-positive nursing staff were infected from the same source and that two EHEC-positive children treated at the hospital at the time of the outbreak, were not.

In conclusion we propose a routinely performed screening for EHEC using PCR for patients suffering from diarrhoea. In cases of an epidemic outbreak a trained and flexible organization is important and PFGE is a good tool for the epidemiological investigation.

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