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An international outbreak of Vero cytotoxin-producing *Escherichia coli* O157 infection amongst tourists; a challenge for the European infectious disease surveillance network

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SUMMARY

In March 1997, an outbreak of Vero cytotoxin-producing *Escherichi coli* O157 (VTEC) infection occurred amongst holidaymakers returning from Fuerteventura, Canary Islands. For the investigation, a confirmed case was an individual staying in Fuerteventura during March 1997, with either *E. coli* O157 VTEC isolated in stool, HUS or serological evidence of recent infection; a probable case was an individual with bloody diarrhoea without laboratory confirmation. Local and Europe-wide active case finding was undertaken through national centres, Salm-Net and the European Programme of Intervention Epidemiology, followed by a case-control study.

Fourteen confirmed and one probable case were identified from England (7), Finland (5), Wales (1), Sweden (1) and Denmark (1) staying in four hotels. Three of the four hotels were supplied with water from a private well which appeared to be the probable vehicle of transmission. The case-control study showed illness was associated with consumption of raw vegetables (OR 8·4, 95% CI 1·5–48·2) which may have been washed in well water.

This investigation shows the importance of international collaboration in the detection and investigation of clusters of enteric infection.

INTRODUCTION

Vero cytotoxin-producing *E. coli* O157 (VTEC) is enzootic in several animal species including cattle, sheep, goats and deer [1]. However, it was only first recognized as a serious human pathogen in 1982 [2], although it has since been increasingly implicated in the aetiology of both outbreaks and sporadic cases of diarrhoea [3], haemorrhagic colitis and post-diarrhoeal haemolytic uraemic syndrome (HUS) [1].

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It has now been linked to more than 100 outbreaks, mainly in the USA [4] and UK [5], but also elsewhere in Europe and the Far East [4]. The majority of outbreaks have been foodborne, mainly associated with the consumption of contaminated, undercooked beef products [1, 4]; but other meat products [6, 7], milk [8, 9], cheese [10], apple cider [11], yogurt [12] and handling contaminated raw potato [13] have also been implicated. Less commonly, outbreaks of VTEC infection have been associated with drinking contaminated mains water [14, 15] or swimming in lakes [16] and paddling pools [17, 18]. No outbreaks of VTEC involving cases resident in several countries ('international') have been published to our knowledge.

Meta-surveillance has been defined as the pooling of data from national surveillance centres for the purpose of improving the detection of clusters of infection or to help recognize international trends [19]. As part of a European strategy of infectious disease 'meta-surveillance' and control, disease specific networks are being created such as for enteric infections (Salm-Net) [20]. Furthermore, in 1995 the European Programme of Intervention Epidemiology Training (EPIET) was established, with the aim of developing a European network of public health epidemiologists trained in field epidemiology to further strengthen European surveillance [21].

On 24 March 1997, the Finnish public health authorities were informed of two cases of HUS occurring in $1\frac{1}{2}$- and 5-year-old Finnish children. They presented to hospital with acute renal failure on 19 March, having recently returned from vacation with their families. They had been staying in the same hotel between 6 and 19 of March, in the resort of Corralejo on Fuerteventura, one of the Canary Islands. VTEC infection, which is notifiable in Finland (unlike HUS) was suspected as the underlying aetiology of their illness.

Corralejo (population 5235) is situated on the Northern tip of the island of Fuerteventura, a popular tourist destination. The town has 43 hotel complexes, 72 restaurants and 1 health care centre. No cases of HUS or O157 VTEC had been notified to the Spanish authorities from the island prior to these tourist cases.

Local and international outbreak investigations were conducted to determine the magnitude of the outbreak, the source or vehicle of infection and to enable the implementation of timely and effective disease control measures.

METHODS

Epidemiological investigation

Case finding was initially undertaken with a confirmed case being defined as an individual who had stayed in Fuerteventura during March 1997 and had any one of the following: *E. coli* O157 VTEC isolated in stool, clinical HUS or serological evidence of recent *E. coli* O157 VTEC infection. A probable case was defined as an individual who had stayed in Fuerteventura during March 1997 who presented with bloody diarrhoea with no laboratory confirmation of *E. coli* O157 VTEC infection.

Case searching was undertaken on several levels. In Finland, the microbiological laboratories and hospitals were informed of possible cases amongst returning travellers. Finnish tourists who had visited the island during March 1997 were contacted directly through their travel agents and asked about their symptoms during and after their vacation. Those who complained of recent diarrhoea were requested to provide a stool and serum sample. The Spanish local authorities were informed of the cases in returning travellers. They initiated a local investigation on 25 March, consisting of an active case search from general practitioners, health centres and hospitals for all individuals presenting with diarrhoea since 1 March 1997. As tourists from many other European countries had been staying on the island, active case searching was also undertaken through Salm-Net and the network of EPIET fellows, resident in various national surveillance centres throughout Europe. Identified cases were interviewed by researchers (usually the EPIET fellow) from each national centre and information collected about demographics, symptoms and potential exposures in the 10 days prior to disease onset.

Case-control study

After initial in-depth interviews and environmental observations, a preliminary hypothesis was developed of disease acquisition from exposure to contaminated pipe-water, originally derived from a private well. A case-control study was thus conducted to test the hypothesis. A control was defined as either an asymptomatic or seronegative person who had travelled to Fuerteventura, but did not belong to the family of a case. Lists of travellers were obtained from travel agencies in each country where cases were

resident. Three controls were chosen for each case and were stratified by hotel, time of vacation, nationality and age (within 10 years for adults and within 5 years for children). They were systematically sampled from travel agency lists.

A questionnaire was administered by telephone in the local language to both cases and controls by researchers at each national surveillance centre. The questionnaire collected information about potential exposures during their stay including swimming, showering, diving, swallowing water, consumption of various food items, excursions, and restaurants visited.

Environmental investigation

This was undertaken by the local authorities. The origin of water supplies to the various hotels was determined. No sampling of water or food specimens was undertaken for microbiological examination.

Microbiological methods

Faecal samples were cultured for *E. coli* O157 VTEC on sorbitol MacConkey agar (SMAC) and incubated at 37 °C for 24 h. Non-sorbitol fermenting colonies were selected and tested by slide agglutination using antibodies to *E. coli* O157 VTEC. Presumptive *E. coli* O157 VTEC isolates were serotyped, phage-typed and examined for genes encoding Verocytotoxin [22–24] by the Laboratory of Enteric Pathogens (LEP), CPHL, Colindale. Sera were examined for antibodies to the LPS of *E. coli* O157 VTEC by ELISA and immunoblotting procedures [25–27]. Nine samples of *E. coli* O157 VTEC submitted to LEP were subtyped by DNA-based methods. Strains were tested by PCR for the subtype of VT2 genes carried and were compared by pulsed field gel electrophoresis (PFGE) of *Xba*I digests of their genomic DNA [28].

Statistical methods

Calculations were performed with Epi-Info (v. 6.04a, Centers for Disease Control, Atlanta, USA). Statistical tests used a 5% ($P < 0{\cdot}05$) level of significance throughout. Controls were stratified with cases on week of arrival, nationality and age group (as indicated above) to form eight strata. Weighted Mantel–Haenszel odds ratios with 95% confidence intervals for each of the possible risk factors were calculated combining the OR from these strata. The adult Danish controls were excluded from this initial analysis, as the infant Danish case had stayed in a different hotel. All cases and controls were included in the final logistic regression analysis (Stata v. 3.1, Stata Corp. Texas, USA) using a logistic regression model adjusting for age, sex and hotel. All potential risk factors reaching significance at the 20% ($P < 0{\cdot}20$) level were entered. Due to the limits set out by the size of the dataset, negatively associated factors were removed from the model.

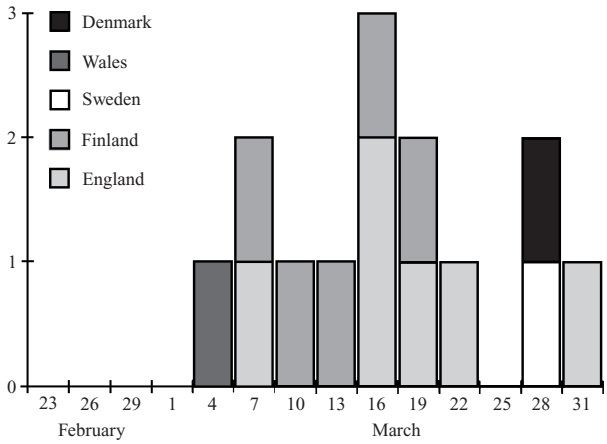

Fig. 1. Cases of *E. coli* O157 VTEC ($n = 14$) by date of symptom onset and country of origin among travellers to Fuerteventura, Spring 1997.

RESULTS

Epidemiological investigation

Fourteen confirmed and one probable case were identified, all of whom were tourists. The cases were of 5 nationalities: 7 (47%) from England, 5 (33%) from Finland, 1 from Wales, 1 from Denmark and 1 from Sweden. No cases were identified amongst local residents. Five of the cases were children under 5 years of age and the remainder of the cases were adult (median age of all cases 45 years; range 6 months to 77 years). Nine cases (60%) were female. The cases stayed in four hotels in the resort of Corralejo between 19 Feb and 6 April 1997.

The cases fell unwell between 4 March and 1 April 1997 (Fig. 1), with the date of onset unknown for one case. All 15 cases experienced diarrhoea, of whom 8 (53%) reported blood in their stools, 7 (47%) fever and 12 (80%) abdominal pain. The median duration of illness was 10 days (range 2–28 days). Twelve (80%) consulted a doctor and 6 (40%) were hospitalized. Three (20%) developed HUS.

E. coli O157 VTEC was isolated from the stools of nine cases. All *E. coli* O157 VTEC isolates were phage

Table 1. *Potential risk factors for* E. coli *O157 VTEC associated infection amongst cases and controls by univariate analysis, Fuerteventura, Spring 1997*

Exposure	Cases ($n = 12$) No. (%)	Controls ($n = 32$) No. (%)	OR_{MH}	95% CI
Swim	8 (67)	18 (56)	1·5	0·3–7·4
Swallow	3 (25)	4 (13)	1·9	0·3–10
Bath	8 (67)	16 (50)	1·8	0·4–7·4
Shower	9 (75)	28 (88)	0·3	0·0–2·3
Market	8 (67)	8 (25)	10·1	1·5–67·9
Raw vegetables	8 (67)	10 (31)	3·8	0·8–16·7

type 2, Vero cytotoxin type 2 with identical antimicrobial resistance patterns (streptomycin, sulphonamides, tetracycline). Of the nine strains submitted to LEP, all possessed both the VT2 and VT2c sequences. Eight strains were indistinguishable by PFGE and the remaining strain had a single extra fragment. Four cases had only serological evidence of infection, including one case with HUS. One of the original Finnish HUS cases had no serological or microbiological confirmation of infection. The one probable case was stool culture negative, but had been given antibiotics prior to sampling.

Initial detailed case interviews revealed no food source or eating pattern common amongst cases, however, many cases had used the hotel swimming pools, showers and baths.

Case-control study

Twelve of the cases (3 further cases were detected after the case-control study had been completed) and 37 controls were recruited for the case-control study. Of these controls, 24 were from England and Wales, 6 from Finland, 5 from Denmark and 2 from Sweden. Fifteen controls (41%) were male compared to 6 (50%) cases. The median age of cases was 36 years and of controls 34 years (range 2–63 years). The median length of stay on the island was 7 days for both cases (range 4–21 days) and controls (4–14 days).

A large proportion of cases swam in the hotel pools, and bathed or showered in the hotels (Table 1). However on stratified single variable analysis, cases were no more likely than controls to have been exposed to these risk factors (Table 1). Shopping at one local supermarket was significantly associated with infection (OR_{MH} 10·1, 95% CI 1·5–67·9). However, examination by hotel strata revealed that in one hotel where two cases had stayed neither reported using the supermarket, whereas in another hotel most of the cases had used the supermarket compared to controls (Table 2). Consumption of any individual food item including meat products was not significantly associated with infection, although a large proportion of cases had eaten raw vegetables (OR_{MH} 3·8, 95% CI 0·8–16·7).

Table 2. *Distribution and shopping habits of cases of* E. coli *O157 VTEC and controls according to hotel, Fuerteventura, Spring 1997*

	Proportion shopping at local supermarket	
	Cases	Controls
Hotel 1	3/3	8/9
Hotel 2	5/7	4/20
Hotel 3	0/2	0/7

In the logistic regression model adjusting for age, hotel and sex, the only independent risk factors associated with infection were consumption of raw vegetables prepared in the hotel (OR_{adj} 8·4, 95% CI 1·5–48·2) and shopping at one local supermarket (OR_{adj} 19·1, 95% CI 1·9–195·3).

Environmental investigations

The main water source on the island was desalinated water supplied to hotels or private homes either by pipe or tanker. A small number of private wells also provided water. Although the desalinated water was potable, due to the salty taste, most residents and visitors to the island drank bottled water and used pipe-water for personal use.

Three hotels, where 14 of the 15 cases stayed, all received untreated water from a single private well

situated outside the town. None of the other 40 hotels in the resort received water from this well. Only a single case occurred in these other hotels. The water was delivered by tanker either daily or on alternate days and was stored in large on-site underground reservoirs. The water was then distributed by pipe for use in the hotel showers, taps and swimming pools. The well was found to be poorly maintained and partially open with livestock grazing nearby. However, no water or animal specimens were taken. No previous records of microbiological monitoring of the well water were available. It was closed on 29 March. Only one case fell ill after this date, 3 days later on 1 April.

DISCUSSION

This report describes an international outbreak of *E. coli* O157 VTEC infection amongst tourists of several nationalities who had stayed in four hotels in the resort of Corralejo, Fuerteventura, Spain.

There were several limitations associated with the study. Case-finding relied upon national surveillance systems, the quality of which varies dramatically [29]. There were tourists from several other European countries in Fuerteventura and although case finding was undertaken in other European countries, there was probably international differential case-ascertainment [29]. Furthermore, the small total number of cases limited the power of the case-control study to detect a significant association. The relatively long delay of 2–3 months in implementing the case-control study decreased the quality of exposure information obtained and probably resulted in a degree of differential recall bias between cases and controls. Stratifying of cases and controls for several variables such as nationality in the study design although theoretically controlling for several confounding factors, also lead to problems in selecting appropriate controls and thus zero frequency in some strata. Furthermore, the lack of environmental sampling of water, food or livestock, meant the conclusions relied purely on the epidemiological investigation.

Several pieces of evidence suggested the vehicle of transmission was water from a local private well. First, 14 of the 15 cases stayed in 3 of the 43 resort hotels, all of which were supplied from the same well. None of the other hotels in the resort were supplied with water by this well. Only one case (stool culture negative but serologically confirmed) stayed in one of the other hotels. Secondly, all cases occurred with onset dates compatible with exposure up to the time of closure of the well, including the case who fell ill 3 days after the well was closed as the usual incubation period of O157 VTEC is 2–10 days. Thirdly, no cases were detected amongst the local population who reportedly did not use the well. Fourthly, although the case control study did not demonstrate a direct association between illness and water exposure, it did show an association between illness and eating raw vegetables and also shopping at the local market. No other food item, including meat products were implicated as a potential vehicle for infection. The increased risk associated with shopping at the local supermarket, could have been due to these individuals washing purchased food such as raw vegetables in their rooms with tap water. Finally, none of the cases staying at one hotel reported using the supermarket suggesting this was not the source.

What are the public health implications for visitors? The vast majority of tap water for public use is supplied via pipes or tankers from desalination plants. Private wells only provide a small proportion of the piped water on the island. Although piped water is supposed to be potable, most residents and tourists routinely drink and cook with bottled water, due to the salty taste of the piped-water. Tap water generally only being used for personal hygiene. The study does seem to suggest a need for monitoring piped water quality particularly if derived from private wells.

One positive feature of this outbreak investigation was the active communication and collaboration between the involved parties, facilitated by Salm-Net and EPIET. This resulted in a prompt local and international investigation, with early recognition of the outbreak and a public health intervention, involving closure of the suspected contaminated water source only 5 days after notification of the two Finnish cases. In addition, these networks made possible case finding throughout the EU and the implementation of a multi-centred case-control study. The delays in implementing the case-control study were in part due to the need to translate the designed questionnaire into other languages, followed by the collation and analysis of the data. Building up a stock of questionnaires related to different exposures in different languages of the EU would perhaps allow a more rapid construction of a shared questionnaire in such situations. The conduct of such international analytical studies is in its infancy but is likely to become increasingly important in the EU with the increased movement of people and goods. The need

for surveillance networks dedicated to detecting clusters of linked infectious intestinal disease including O157 VTEC cases, which are either travel related or due to a widely distributed vehicle is also increasingly apparent. This involves the need for common subtyping techniques and also the collection and collation of epidemiological information from individual cases. The establishment of Salm-Net and its extension in the form of Enter-net to include the surveillance of VTEC are valuable steps forwards.

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