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Outbreaks of food poisoning in adults due to *Escherichia coli* O111 and campylobacter associated with coach trips to northern France

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SUMMARY

Thirty-seven out of 48 people on a coach excursion to northern France developed gastrointestinal symptoms within 4 days of the trip. Twenty-six had stool samples positive for *Escherichia coli* O111, 8 were also positive for *Campylobacter* species, and 1 was positive for campylobacter alone. Strains of *E. coli* were positive for the effacing and attaching protein (*eaeA*) gene, but negative for other *E. coli* virulence genes, and therefore belonged to the enteropathogenic *E. coli* (EPEC) group. Twenty-two out of 37 people in a second party which followed the same itinerary 2 weeks later also became ill. One had a stool sample positive for *E. coli* O111. Analytical epidemiology suggested that the source of infections was a restaurant in northern France at which both parties had eaten.

INTRODUCTION

In the past, *E. coli* O111 has been reported as a causative organism for outbreaks of diarrhoea in infants in the United Kingdom [1, 2], as well as in the United States [3]. In addition, it has been reported as a cause of illness in both adults and children in Finland [4], and cases of haemolytic uraemic syndrome (HUS) associated with infection by Vero cytotoxin producing strains of *E. coli* O111 have been reported in France [5] and Australia [6]. However it has not previously been reported as a cause of food poisoning outbreaks in adults in the United Kingdom [7]. We report two outbreaks of illness attributable to *E. coli* O111 which appear to have been imported to the United Kingdom from northern France.

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OUTBREAKS AND INVESTIGATIONS

In November and December 1995 the Public and Environmental Health Department of Sheffield City Council were informed of two outbreaks of illness among people who had been on two separate weekend coach trips, organized by the same tour operator, to northern France.

A party of 48 people travelled from Sheffield over the weekend of 10–12 November 1995 (Party 1). After leaving Sheffield on the Friday afternoon, they ate at a motorway service station that evening, and had a late night buffet at a hotel in Folkestone, Kent, where they stayed the night. On the Saturday they travelled to France, where they ate lunch at a restaurant, and returned to Folkestone that afternoon. Breakfast and evening buffet were eaten in the hotel, as were breakfast and lunch on the Sunday. They returned to Sheffield, again stopping at a motorway service station on the return journey, on the Sunday afternoon.

Table 1. *Numbers of people reporting symptoms*

	Number of cases	Nausea	Diarrhoea	Vomiting	Abdominal pain	Blood in stools	Fever	Headache	Malaise	Body aches	Chills
Party 1	37	24	30	21	24	1	15	19	23	16	14
Party 2	22	15	15	14	13	0	8	11	14	11	6

One member of the party became ill on the Saturday (at 20.00), others on the Sunday and Tuesday. The commonest symptoms were diarrhoea, nausea, vomiting and abdominal pain. In addition some people suffered symptoms of fever and headache, and one person reported blood in the stools (Table 1). Symptoms were self limiting and none lasted more than 72 h.

Two weeks later another coach party (Party 2), with 37 members, organized by the same travel company, followed the same itinerary. A number of these also became ill, though these cases were only brought to the attention of the authorities 10 days after the event. The nature, time course and outcome of symptoms were similar to Party 1.

Cases were defined as those people suffering diarrhoea, vomiting or 'flu-like symptoms within 4 days of the trip. A food history questionnaire, detailing all food items consumed during the weekend (87 items for Party 1, 92 for Party 2) was posted to all members of each coach party.

The hotel in Folkestone was inspected by local Environmental Health Officers, but no cause for concern was found. The relevant local authority in northern France was contacted. Initially investigations were hampered by public sector strikes. Later, inspectors visited the restaurant at which the party had eaten, but as it was closed were not able to inspect the premises.

Isolation and identification of *E. coli* O111

Faecal samples were obtained from 44 of the 48 members of Party 1 and from 18 of the 37 in Party 2. Samples were examined by standard methods for salmonellae, shigellae, *Cryptosporidium* and campylobacter and by both direct culture onto cefixime tellurite sorbitol MacConkey agar [8] and an immunomagnetic separation technique [9] for *E. coli* O157. Samples were examined for viruses using standard electron microscopy.

Cultures of samples plated onto desoxycholate citrate agar (CM35, Unipath, Basingstoke) after incubation at 37 °C for 18 h yielded lactose non-fermenting colonies, which were tested by a slide agglutination method for *E. coli* O111. Isolates that gave a positive slide agglutination test were confirmed as *E. coli* using biochemical tests as described previously [10] and confirmed as serogroup O111 by agglutination to titre with antiserum to *E. coli* O111 (Laboratory for Microbiological Reagents, Central Public Health Laboratory, Colindale, London).

Detection of virulence mechanisms

Vero cytotoxigenicity was determined by Vero cell culture assay [10]. Strains were examined for the presence of virulence genes which may be found in *E. coli*: Vero cytotoxins 1 and 2 (VT_1, VT_2), heat-labile enterotoxin (LT), heat-stable enterotoxins (ST1a and ST1b), effacing and attaching protein (*eaeA*), chromosomal invasiveness associated locus (*ial*) and invasiveness plasmid antigen H (*ipaH*). Based on gene sequence data from previous studies [11–14], DNA specific for these *E. coli* structural genes was prepared by the polymerase chain reaction, random-prime labelled with digoxigenin-11-dUTP, and used in colony hybridization reactions as described previously [11, 15]. Strains known to be positive and negative for each gene were included as controls.

Plasmid analysis

Plasmids were extracted by an alkaline detergent method and were separated by submerged gel electrophoresis in Tris–acetate–EDTA buffer with agarose 1% stained by ethidium bromide and visualized on an ultraviolet transilluminator [16]. A control *E. coli* K-12 strain (NCTC 50192–39R861) harbouring plasmids of 148, 63·4, 36 and 6·9 kb was included with each batch of tests. For this control strain, log of plasmid size was plotted against distance migrated through the agarose gel and approximate sizes of plasmids from strains of *E. coli* O111 were estimated from this graph.

RESULTS

Thirty-seven of the 48 people from Party 1, and 22 of the 37 people in Party 2 fulfilled the case definition

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Table 2. *Demographic details*

	Male	Female	Average age (range) (years)
Party 1			
Cases	12	25	47 (9–72)
Non-cases	5	6	51 (37–75)
Party 2			
Cases	6	16	58 (45–82)
Non-cases	8	7	58 (21–81)

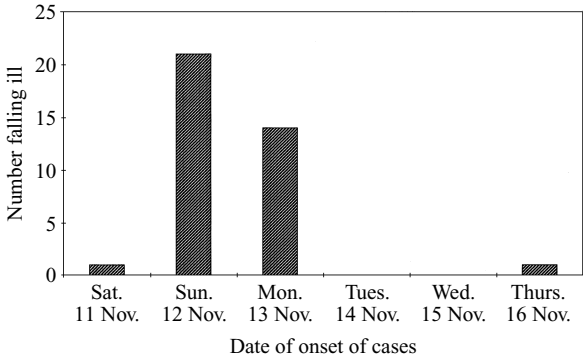

Fig. 1. Epidemic curve for Party 1.

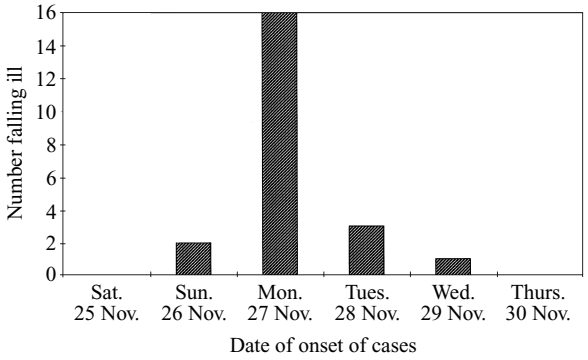

Fig. 2. Epidemic curve for Party 2.

(Table 2). The epidemic curves are shown in Figures 1 (Party 1) and 2 (Party 2).

Forty-seven people from Party 1 completed and returned the food history questionnaire. The relative risk of illness following consumption of each of the 87 food items was calculated, and those with associated *P* values (2 tailed, Fisher's exact) less than 0·1 are detailed in Table 3, together with the confidence intervals, *P* values and attack rates. Only one of these foods was positively associated with illness at $P < 0{\cdot}05$ level, prawn mayonnaise vol au vents on the Saturday evening, with an associated *P* value of 0·022.

All 37 people from Party 2 returned questionnaires. The relative risk of illness following consumption of each of the 92 food items was calculated, and those with associated *P* values (2 tailed, Fisher's exact) less than 0·1 are detailed in Table 4. The lettuce and gherkins were significantly associated with illness. These were served for lunch at the restaurant in France.

Of the 37 people from Party 1 who fulfilled the case definition, 36 had stool samples examined. Twenty-seven of these were positive for *E. coli* O111, of which 8 were also positive for *Campylobacter* species. One person had stools positive for campylobacter but not *E. coli* O111. The relationship between symptoms and microbiological findings is shown in Table 5.

Of the seven people in Party 1 who did not fulfil the case definition, but who had stool samples examined, three were positive for *E. coli* O111, but none for any other pathogen.

Of the 22 people from Party 2 who fulfilled the case definition, 18 had stool samples examined. One of these had been arranged by the patient's general practitioner 5 days after the patient returned from the trip. As a result of the delay in the authorities' becoming aware of these cases, the other samples were not obtained until 11–18 days after the Party had returned. Only the first one gave a positive result, for *E. coli* O111. There were no positive results for campylobacter. No stool samples were available for any members of the party who did not fulfil the case definition.

Salmonellae, shigellae, *Cryptosporidium*, *E. coli* O157 and viruses were not detected in any of the samples from either party.

All isolates of *E. coli* O111 gave the same reaction pattern in a series of 12 biochemical tests. None of the strains produced Vero cytotoxin as determined by Vero cell assay. All were positive for the *eaeA* gene but negative for the VT_1, VT_2, LT, ST1a, ST1b, *ipaH* and *ial* genes. All isolates contained plasmids of 93, 55, 4·8 and 2 kb.

CONTROL MEASURES

The tour operators decided not to send any more coach parties to this restaurant following these two outbreaks. The restaurant itself was shut at the beginning of January 1996, following complaints from several parties (Local Authorities Co-ordinating Body on Food and Trading Standards, personal communication).

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Table 3. *Attack rate, relative risks (and 95% confidence intervals) and associated P values (2 tailed, Fisher's exact) for selected food items, Party 1*

	Ill		Not ill					
Food item	Eaten	Not eaten	Eaten	Not eaten	AR*	RR†		*P*
Prawn mayo vol au vents, Sat. p.m.	14	23	0	10	100·0	1·43	(1·15–1·8)	0·022
Corned beef sandwiches, Fri. night	11	26	0	10	100·0	1·38	(1·13–1·7)	0·088
Coffee, Sun. lunch	11	26	0	10	100·0	1·38	(1·13–1·7)	0·088
Food on return ferry	10	27	0	10	100·0	1·37	(1·13–1·67)	0·091
Corned beef sandwiches, Sat. eve	10	27	0	10	100·0	1·37	(1·13–1·67)	0·091
Tomatoes, Sun. a.m.	26	11	10	0	72·2	0·72	(0·59–0·88)	0·088
Trifle with cream, Sun. lunch	25	12	10	0	71·4	0·71	(0·58–0·88)	0·046
Fried bread, Sun. a.m.	22	15	10	0	68·8	0·69	(0·54–0·87)	0·019
Salad, Sat. lunch	14	23	8	2	63·6	0·69	(0·49–0·97)	0·03
Cream cheese, Sat. lunch	5	32	5	5	50·0	0·58	(0·31–1·09)	0·024
Mushrooms, Sat. a.m.	0	37	3	7	0·0	—	—	0·007
Mushrooms, Sun. a.m.	0	37	2	8	0·0	—	—	0·042

* AR, Attack rate in percent.
† RR, relative risk.

Table 4. *Attack rate, relative risks (and 95% confidence intervals) and associated P values (2 tailed, Fisher's exact) for selected food items, Party 2*

	Ill		Not ill					
Food item	Eaten	Not eaten	Eaten	Not eaten	AR*	RR†		*P*
Lettuce, Sat. lunch	21	1	9	5	70·0	4·2	(0·69–25·52)	0·024
Gherkin, Sat. lunch	15	7	3	11	83·3	2·14	(1·16– 3·96)	0·006
Tea, Sat. a.m.	17	5	6	8	73·9	1·92	(0·93– 3·98)	0·036
Tea, Sun. lunch	16	6	5	9	76·2	1·9	(0·98– 3·7)	0·028
Salami, Sat. lunch	16	6	6	8	72·7	1·7	(0·88– 3·27)	0·073
Olives, Sat. lunch	11	11	3	11	78·6	1·57	(0·95– 2·59)	0·086
Ham sandwiches, Sat. eve	11	11	11	3	50·0	0·64	(0·39– 1·05)	0·086
Ham sandwiches, Fri. night	8	14	10	4	44·4	0·57	(0·32– 1·01)	0·04
Bread, Sun. a.m.	5	17	8	6	38·5	0·52	(0·25– 1·08)	0·036
Veal and ham pie, Fri. night	2	20	5	9	28·6	0·41	(0·13– 1·37)	0·084
Egg mayo vol au vents, Fri. night	2	20	5	9	28·6	0·41	(0·13– 1·37)	0·084

* AR, Attack rate in percent.
† RR, relative risk.

DISCUSSION

The evidence implicating *E. coli* O111 as the causative organism is stronger for the first than for the second coach party. The majority of cases had stool samples positive for this organism, which is unusual, and is know to cause gastrointestinal disease [1–6]. The additional isolation of campylobacter shows that this organism may have contributed to the illnesses in some cases. For the second party, the evidence implicating *E. coli* O111 is more circumstantial, as only one case was positive. However, no other pathogens were isolated (which may have been due to the delay in obtaining samples). Furthermore, the indistinguishable strain of the organism, and the fact that the parties had followed exactly the same itineraries and eaten similar meals, indicates that a common source is highly probable.

Although the majority of people on the coach trips completed and returned food history questionnaires,

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Table 5. *Relationship between faecal microbiology and symptoms – number of individuals with stool sample result (total) who reported each symptom*

	Stool sample result			
Symptom	*E. coli* O111 (19)	Campylobacter (1)	*E. coli* O111 and campylobacter (8)	No pathogen (16)
Nausea	11	—	6	7
Diarrhoea	16	1	7	7
Vomiting	10	1	5	5
Abdominal pain	13	—	4	7
Blood in stools	—	—	—	1
Fever	7	1	3	4
Headache	10	—	3	6
Malaise	9	1	7	7
Body aches	5	—	5	7
Chills	9	1	1	4

analysis was of limited value, as the majority of people on the trip ate the same set menus throughout. The elevated relative risk associated with the consumption of prawn mayonnaise vol au vents in Party 1 is probably a result of chance, as there were 87 individual food items in the questionnaire. In the second party two food items served at the lunch were strongly associated with illness.

Ideally, further evidence that a particular food item was the vehicle of infection would have been forthcoming from analysis of food samples. Unfortunately for these outbreaks this was not the case. Inspection of the hotel in Folkestone revealed nothing which could give grounds for concern. The restaurant in France was not formally inspected, but there was indirect evidence that standards of hygiene were not adequate.

The evidence available suggests, therefore, that the most likely source of infection was the restaurant in northern France which was visited by both coach parties. *E. coli* O111 has previously been reported as a cause of food poisoning outbreaks in France [5]. This organism has not previously been reported as a cause of food poisoning outbreaks in adults in the United Kingdom, though there were 36 sporadic cases reported in 1995 and 11 in the first 6 months of 1996 (Dr T. Cheasty, Laboratory of Enteric Pathogens, Communicable Disease Surveillance Centre).

Faecal samples were examined by standard methods for salmonellae, shigellae and *Cryptosporidium* with negative results. Samples were not initially examined specifically for classical EPEC serogroups as all the patients were adults. However, lactose non-fermenting organisms of similar colonial appearance which were found on many of the DCA plates were subsequently identified as *E. coli*, all having the same biochemical profile. Further investigation showed these to be *E. coli* O111. Presence of the *eaeA* gene and absence of any other virulence genes confirmed the organisms as belonging to the EPEC group.

None of the cases was hospitalized. However, other strains of *E. coli* O111 produce Vero cytotoxin, and may cause HUS [5, 6]. Those involved in communicable disease surveillance and control should be aware of the possibility of imported infections caused by this organism.

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