
An outbreak of infection due to verocytotoxin-producing *Escherichia coli* O157 in four families: the influence of laboratory methods on the outcome of the investigation

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SUMMARY

Three members of family A, who had diarrhoea on 20 October, lived on a small arable farm which had 10 cattle. Manure from the animals was used to fertilize the ground for growing potatoes which were then offered for retail sale, unwashed, directly from the farm. The mother from family B bought potatoes, which were covered with manure, from family A in early November and over the subsequent 10 days she became ill with diarrhoea and her daughter and son both became ill with bloody diarrhoea. The mother from family C visited family B while the daughter from the latter family was symptomatic; the mother developed diarrhoea several days later. The mother and two sons from family D visited family B while the son from the latter family was symptomatic; the first son developed bloody diarrhoea 6 days later which progressed to development of haemolytic-uraemic syndrome. Direct culture of faecal samples onto cefixime rhamnose sorbitol MacConkey agar failed to isolate *E. coli* O157 from any of the symptomatic patients, and direct culture onto cefixime tellurite sorbitol MacConkey agar isolated the organism from only one patient. In contrast, a combination of isolation of *E. coli* O157 by immunomagnetic separation and detection of *E. coli* O157-specific secretory IgA, suggested *E. coli* O157 infection in all eight symptomatic patients, but not in any of the family members who were not ill. Two children who excreted the organism for 60 and 89 days respectively were the only two patients who did not develop a secretory IgA response. *E. coli* O157 was not isolated from potatoes from the farm and faecal samples from the farm animals were not available for examination. The study illustrates the need to use the most sensitive methods available during the investigation and follow up of cases of *E. coli* O157 infection.

INTRODUCTION

Verocytotoxin-producing (VT⁺) *E. coli* (VTEC) cause haemorrhagic colitis (HC), haemolytic-uraemic syndrome (HUS) and occasionally mild non-bloody diarrhoea in man; infections may be asymptomatic [1]. In the UK, VT⁺ *E. coli* O157, the most common serogroup associated with illness in man, has been isolated from cattle [2–5] and beef and beef products, milk and milk products, and vegetables or fruit contaminated with animal manure have been identi-

fied as sources of human infection [3, 4, 6–8]. Person-to-person transmission has also been reported as secondary spread after foodborne infection [9] and in institutions [10, 11].

In October–November 1995 an outbreak of infection due to *E. coli* O157 occurred in three families from the Rotherham area and one family from Bristol. This study assesses the influence of laboratory diagnostic methods on the outcome of the outbreak investigation.

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MATERIALS AND METHODS

Study population

The population studied was three families from the Rotherham area and one family from Bristol. The interaction between the families is summarized in Figure 1.

Family A

Family A lived near Rotherham on a small arable farm which had 10 cows and 1 horse. Manure from the animals was used to fertilize the ground for growing potatoes which were then offered for retail sale, unwashed, directly from the farm. The mother, son and daughter had diarrhoea on 20 October. The father had only a 'mild upset stomach' with no diarrhoea. Faecal samples were submitted to a local laboratory at this time but as the clinical details did not indicate that *E. coli* O157 was a likely cause of their illness, they were not examined for this organism. Faecal samples from all the family were submitted to Sheffield Public Health Laboratory (SPHL) in mid-November. Potatoes (3 kg) were submitted to SPHL in late November. Faecal samples from the animals were not available for examination.

Family B

Family B lived in Rotherham. The mother bought potatoes from the farm of Family A in early November. She could not recall the exact date but did remember that the potatoes were very dirty and covered with manure. The mother developed abdominal pain and diarrhoea without blood on the 3 November, the daughter began with bloody diarrhoea on the 8 November and the son with abdominal pain and bloody diarrhoea on the 12 November. The father was not ill. Faecal samples from all the family were submitted to SPHL during November and at intervals up to the end of February the following year.

Family C

Family C lived in Rotherham. The mother was the sister of the mother in Family B and visited this family on the 8 November while the daughter from family B was symptomatic. The mother developed diarrhoea on the 10 November. The father, son and two daughters did not visit family B and were not ill. Faecal samples from all the family were submitted to SPHL in late November/early December.

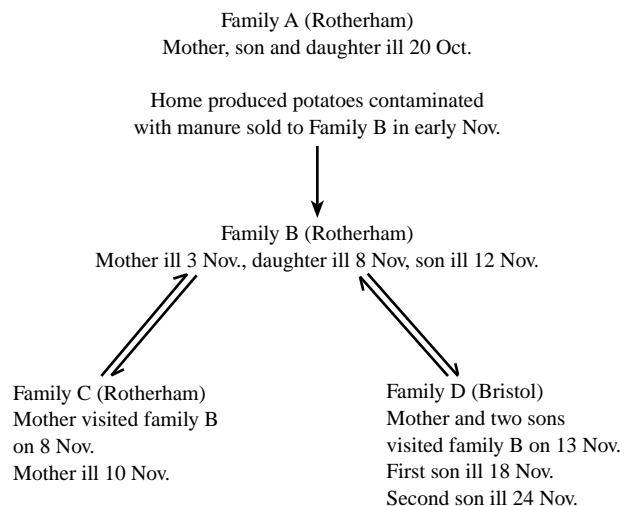


Fig. 1. Interaction between the four families studied.

Family D

Family D lived in Bristol. The mother and two sons visited family B on the weekend of the 12–13 November when the son from family B was symptomatic. The mother was not ill. The first son developed bloody diarrhoea on the 18 November which progressed to development of HUS. He was admitted to hospital in Cheltenham on the 24 November and transferred to Bristol shortly afterwards. A faecal sample was submitted to the local laboratory where it was reported as negative for *E. coli* O157; this sample was later transferred to SPHL via Bristol PHL. The second son developed bloody diarrhoea on the 24 November but a specimen was not sent for microbiological examination. The father and third son did not visit family B and were not ill.

The symptomatic patients from all families made a full recovery from the infection.

Isolation of *E. coli* O157

Direct culture of *E. coli* O157

Approximate 10 μ l volumes of faecal samples were inoculated directly on to cefixime rhamnose sorbitol MacConkey (CR-SMAC) medium [12] and cefixime tellurite sorbitol MacConkey (CT-SMAC) medium [13]. After overnight incubation at 37 °C, apparently sorbitol and rhamnose non-fermenting colonies from CR-SMAC and apparently sorbitol non-fermenting colonies from CT-SMAC were tested for agglutination with a latex test kit (Oxoid – DR622) for detecting *E. coli* O157. Isolates that gave positive

results with this test were further characterized as described below.

Immunomagnetic separation of E. coli O157

Approximately 0.5 g of faecal sample was placed in 5 ml of buffered peptone water (Oxoid – CM509) supplemented with vancomycin 8 mg/l, cefixime 0.05 mg/l, and cefsulodin 10 mg/l (BPW–VCC) [3]. After vortex mixing, broths were incubated at 37 °C for 6 h and 1 ml of broth was then added to 20 µl of magnetic beads coated with an antibody against *E. coli* O157 (Dynabeads anti-*E. coli* O157; Dynal, Oslo) in a 1.5 ml microcentrifuge tube. The beads were suspended evenly in the broth culture by vortex mixing and were placed in a rotating mixer so that they were mixed by inversion every 2–3 sec for 30 min at ambient temperature. Tubes were placed in a magnetic separator rack (MPC-10, Dynal, Oslo) and the magnets were placed in position and left for 5 min. The culture supernate was removed by aspiration with a pasteur pipette, the magnetic slide was removed from the rack, the beads washed by resuspension in 1 ml of PBS pH 7.2 with Tween-20 0.05% v/v (PBST) and the magnetic slide replaced for 2 min. The beads were washed in PBST in this way once more, the magnetic slide replaced for 2 min, the supernate removed and the beads resuspended in *c.* 25 µl of PBS. Beads were inoculated on to CT-SMAC medium and incubated overnight at 37 °C. Sorbitol non-fermenting colonies were examined as above. Apparently sorbitol non-fermenting colonies which did not agglutinate with the latex test kit for *E. coli* O157 were identified by a standard series of biochemical tests [2].

Potatoes

Surface soiling from the potatoes was washed off into 4 × 225 ml of BPW–VCC which were incubated at 37 °C for 6 h. IMS and culture of *E. coli* O157 was then performed as above.

Characterization of isolates

Identification

As described previously [2], isolates that gave a positive latex test result were confirmed as *E. coli* by biochemical tests and confirmed as serogroup O157 by agglutination to titre with antiserum to *E. coli* O157 (Laboratory for Microbiological Reagents,

Central Public Health Laboratory, 61 Colindale Avenue, London).

Verocytotoxin production

Verocytotoxigenicity was determined by Vero cell culture assay [2] and toxin type by specific hybridization with DNA probes for the VT₁ and VT₂ genes. Presence of the *eaeA* gene was also determined by DNA hybridization. Using published sequence data [14, 15] DNA specific for the A cistrons of the VT₁ and VT₂ genes, and for the *eaeA* gene, was prepared and labelled with digoxigenin-11-dUTP by the polymerase chain reaction and used in colony hybridization reactions [3, 16]. Known VT₁⁺, VT₂⁺, VT⁻, *eaeA*⁺ and *eaeA*⁻ strains were included as controls in each batch of tests.

Plasmid analysis

Plasmids were extracted by an alkaline detergent method [17] 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. 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 and used to estimate size of plasmids.

Pulsed field gel electrophoresis

Pulsed field gel electrophoresis (PFGE) was performed as described by Thomson-Carter and colleagues [18]. Bacterial cells suspended in chromosomal grade agarose (Bio-Rad) plugs were sequentially treated with lysozyme, RNase, proteinase K, phenylmethylsulphonyl fluoride and Tris-EDTA buffer before being digested with *Xba*I 30U at 37 °C for 6 h. PFGE was performed in a CHEF DR-II apparatus (Bio-Rad) with 1% agarose gels in Tris-borate-EDTA buffer. Gels were electrophoresed for 22 h at 14 °C at a constant 6 volts/cm and with pulse times of 5–50 sec with linear ramping. Gels were stained with ethidium bromide and visualized on an ultraviolet light transilluminator.

Phage typing

All *E. coli* O157 isolates were phage typed by the Laboratory for Enteric Pathogens, Central Public Health Laboratory.

Serology

Sample preparation

Faecal IgA was estimated as described previously [19]. A 10% w/v suspension of faecal sample in PBS with Tween-20 0.5% v/v (PBST) was centrifuged at 13000 g for 10 min; the supernate was removed to a clean tube and stored at -70 °C until required for serological testing.

Antigen preparation

Cells from overnight cultures of *E. coli* O157 strain P737 on blood agar were harvested into distilled water, formaldehyde added to c. 1% w/v, and incubated overnight at 4 °C. Cells were washed three times by centrifuging at 3000 g for 10 min and resuspending in fresh distilled water, before being centrifuged as above and resuspended in 25 mM Tris-HCl pH 8.0 with 100 mM NaCl containing hydrogen peroxide 2% v/v. After incubation at ambient temperature for 30 min, cells were washed a further three times in distilled water and suspended in carbonate buffer pH 9.6.

EIA procedure

Cell suspension was diluted in carbonate buffer pH 9.6 to an OD₆₀₀ of 0.08 and 100 µl of suspension added to the wells of a standard 96 well EIA plate, with buffer only being in wells to be used as controls. After shaking for 2 min and incubation at ambient temperature for 1 h, plates were incubated overnight at 4 °C, and wells washed five times in PBST. Any uncoated sites were blocked by adding 200 µl of PBST with bovine serum albumin (BSA) 1% w/v, shaking briefly, incubating at ambient temperature for 1 h and washing five times as above. Faecal samples were tested in duplicate at a dilution of 100 in PBST with BSA 1% w/v. The following were added in sequence, plates being shaken briefly, incubated at ambient temperature for 2 h and washed five times in PBST after addition: 100 µl of the dilution of faeces, with control wells having only 100 µl PBST with BSA 1% w/v; 100 µl of HRP-conjugated rabbit anti-human IgG or IgA (Dako) at dilutions recommended by the supplier. Freshly prepared chromogenic substrate solution (3,3',5,5'-tetramethylbenzidine in dimethyl sulphoxide) was added and OD₄₅₀ readings recorded. A corrected OD₄₅₀ for each sample was calculated by subtracting the mean reading of the negative control wells from the mean reading of the two sample wells.

RESULTS

The results of examination of the faecal samples for *E. coli* O157 and for IgA antibodies are shown in Table 1. By direct culture onto CR-SMAC, *E. coli* O157 was not isolated from any of the eight symptomatic patients, but the organism was isolated from one patient by direct culture onto CT-SMAC. *E. coli* O157 was isolated from four patients by IMS and six patients were positive for *E. coli* O157-specific faecal IgA; either IMS or IgA, or both, were positive in all eight symptomatic patients. The daughter and son from family B excreted the organism for 60 and 89 days, respectively, and were the only two patients who failed to develop a faecal IgA response. All isolates of *E. coli* O157 were phage type 2, hybridized with probes for *eaeA* and VT₂ but not with one for VT₁, harboured a single 92 kb plasmid and gave indistinguishable banding patterns by PFGE. *E. coli* O157 was not isolated, nor was *E. coli* O157-specific IgA demonstrated, in samples from six family members who were not ill. *E. coli* O157 was not isolated from the potato samples.

DISCUSSION

In October/November a cluster of cases of *E. coli* O157 infection occurred in four families. Several weeks elapsed before epidemiological investigations suggested a putative link between the four families and this almost certainly had an adverse effect on the laboratory investigations.

Three of four members of family A had been ill in October and although *E. coli* O157 was not isolated from faecal samples from these patients submitted over a month later, *E. coli* O157-specific IgA was demonstrated in all three samples. In an outbreak of infection in the E. Midlands in 1982/3 the handling, but not consumption, of contaminated potatoes was identified statistically as a risk factor for infection [20]. In this study, manure-contaminated potatoes supplied from family A were suspected as a possible source of infection for family B. Potatoes examined some 5-6 weeks after the initial case of infection were negative for *E. coli* O157. However the surface contamination by this time was thoroughly dry and this may have adversely affected the survival of any contaminating *E. coli* O157; although the organism may survive for long periods in bovine faeces whilst moist, viability is rapidly lost on drying [21].

Dates of onset of symptoms within families B, C and D suggest that the infection was transmitted

Table 1. Isolation of *E. coli* O157 and detection of specific faecal IgA in specimens from the four families

Patient	Onset date	Sample date	Culture of <i>E. coli</i> O157			
			Direct*			Faecal IgA
			CR	CT	IMS	
Family A						
Mother	24 Oct	30 Nov	—	—	—	+
Son	24 Oct	30 Nov	—	—	—	+
Daughter	24 Oct	30 Nov	—	—	—	+
Father	Not ill	30 Nov	—	—	—	—
Family B						
Mother	3 Nov	29 Nov	—	—	+	+
		16 Dec	—	—	—	+
		12 Jan†	—	—	—	—
Daughter	8 Nov	29 Nov	—	—	+	—
		15 Dec	—	—	+	—
		6 Jan†	—	—	—	—
Son	12 Nov	29 Nov	—	+	+	—
		5 Jan	—	+	+	—
		12 Jan	—	+	+	—
		30 Jan	—	—	+	—
		8 Feb	—	—	+	—
27 Feb†	—	—	—	—		
Father	Not ill	29 Nov†	—	—	—	—
Family C						
Mother	10 Nov	30 Nov	—	—	+	+
Father	Not ill	9 Dec	—	—	—	—
Son	Not ill	9 Dec	—	—	—	—
Daughter 1	Not ill	9 Dec	—	—	—	—
Daughter 2	Not ill	9 Dec	—	—	—	—
Family D						
Son 1	18 Nov	30 Nov	—	—	—	+

* CR = CR-SMAC, CT = CT-SMAC.

† Subsequent negative sample not shown.

person-to-person, as has been documented previously [9–11]. *E. coli* O157 was not isolated from any patients by direct culture onto CR-SMAC and if direct culture onto this medium, or onto plain sorbitol MacConkey (SMAC) agar, had been used as the sole diagnostic test, then this cluster of cases would have been missed. Mother, daughter and son from family B were ill, but by direct culture onto CT-SMAC, *E. coli* O157 was isolated from only the son. However, *E. coli* O157 was isolated by IMS from all three patients, with the mother also being positive for *E. coli* O157-specific faecal IgA. Subsequent monitoring of the daughter and son by IMS and by faecal IgA revealed that, despite being symptomatic for less than a week, they excreted *E. coli* O157 for at least 60 and 89 days, respectively, and that neither child developed a faecal

IgA response. Further work is needed to test the hypothesis that this prolonged excretion may be due to failure to produce specific secretory antibody.

Reports on the length of the period of excretion of *E. coli* O157 following infection differ widely and probably reflect differences both in methodology and in populations studied. According to Tarr and colleagues [22] patients rapidly stop excreting the organism after illness, as determined by direct culture onto plain SMAC, whereas Karch and colleagues [23] used DNA hybridization to detect shedding of the organism for up to 62 days in HC patients and for up to 124 days in HUS patients; excretion of the organism may also be prolonged in young children [24, 25]. IMS was used in the present study and we have previously shown this to be more sensitive than

direct culture for the isolation of *E. coli* O157 from human faecal samples from several different categories of patients [26]; this has subsequently been confirmed by others [27, 28].

Others [29, 30] have reported detection of serum IgM antibodies against lipopolysaccharide (LPS) purified from *E. coli* O157 as a potentially useful diagnostic test in HUS caused by this organism. However, the procedure for LPS purification and subsequent immunoblotting are both time consuming and expensive; the detection of such antibodies is also of doubtful value in uncomplicated diarrhoea or HC due to *E. coli* O157 [19]. In contrast, detection of specific secretory IgA by an enzyme immunoassay based on a whole cell antigen has been shown to be a useful adjunct to diagnosis in diarrhoea and HC caused by *E. coli* O157 [19] and in diarrhoea caused by other organisms [31]; the secretory IgA assay also offers the advantages of being economical and simple to perform on faecal samples already submitted for microbiological examination. In view of this, it would perhaps have been difficult to justify obtaining blood samples from young children and asymptomatic adults specifically for the purposes of this investigation.

This study illustrates the need to use the most sensitive methods available during the investigation and follow up of cases of *E. coli* O157 infection. Although the majority of cases of *E. coli* O157 probably occur after consumption of contaminated beef products or unpasteurized dairy products, the role of contaminated vegetable crops in the transmission of the organism to man warrants further study.

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