The use of serodiagnosis in the retrospective investigation of a nursery outbreak associated with *Escherichia coli* O157:H7

T Cheasty, R Robertson, H Chart, P Mannion, Q Syed, R Garvey, B Rowe

Abstract

**Aims**—To use serology to investigate an outbreak of verocytotoxin (VT) producing *Escherichia coli* O157 in a hospital nursery, following the detection of faecal *E coli* O157 (phage type 49) producing VT type 2.

**Methods**—ELISA and immunoblotting techniques, based on lipopolysaccharide (LPS) purified from *E coli* O157; diagnostic bacteriology; serotyping and phage typing; DNA probes for VT.

**Results**—29 of 126 sera contained antibodies to the LPS of *E coli* O157: 10 were from children, three were from staff, and 11 were from hospital kitchen staff. Five parents of children attending the nursery were antibody positive. Sixty four sera from other hospital staff and controls did not contain antibodies to the LPS of *E coli* O157.

**Conclusions**—Serology detected evidence of infection with *E coli* O157 in 23% of sera examined. By bacteriology alone, only a single case of infection with *E coli* O157 would have been detected. Serology is valuable in providing evidence of infection with *E coli* O157.


**Keywords**: *Escherichia coli* O157; serodiagnosis; outbreak

Strains of *Escherichia coli* producing verocytotoxin (VTEC), and particularly those belonging to serogroup O157, are a major cause of haemorrhagic colitis and the haemolytic–uraemic syndrome in England and Wales as well as in other countries. Infection with *E coli* O157 is an important cause of kidney failure in infants and young children, and fatal cases occur among both the young and the elderly. The first confirmed outbreak of VTEC infection in the United Kingdom, caused by *E coli* O157, was reported in 1983, and since then the numbers of outbreaks and sporadic cases in England and Wales have increased. Between 1992 and 1994 there were 1266 bacteriologically confirmed infections caused by *E coli* O157 and 18 outbreaks. In 1996 there were 660 confirmed infections with *E coli* O157 and at least 10 outbreaks (Laboratory of Enteric Pathogens, unpublished data). Infection with O157 VTEC is most commonly caused by the consumption of foods of bovine origin, predominantly ground (minced) beef and unpasteurised milk, although various other food vehicles including fermented meat products, unpasteurised apple juice, and raw vegetables have been reported. Water borne outbreaks associated with both drinking and recreational water have also been described.

Furthermore, there is evidence that infection can be acquired by person to person spread and by contact with farm animals. The isolation of *E coli* O157 from a patient's stools identifies the cause of haemolytic–uraemic syndrome or haemorrhagic colitis; however, in the absence of a culturable pathogen, particularly late on in the disease, an alternative method of providing evidence of infection is necessary. Infections with *E coli* O157 result in the production of serum antibodies to the lipopolysaccharide (LPS) of this organism and a serodiagnostic test using the purified LPS of *E coli* O157 can provide evidence of infection. Initial characterisation of this immune response showed that it was an IgM rather than an IgG antibody response. Circulating antibody is detectable for at least 74 days after infection. We used this test to investigate a nursery outbreak of infection associated with *E coli* O157.

The outbreak

The outbreak occurred in a nursery which was attached to a hospital and provided care for the children belonging to the hospital staff. The nursery had 11 staff who looked after 31 children overall, with a maximum of 25 during any one period. All nursery meals were provided by the hospital kitchens. In October 1994 two of the children who attended the nursery developed the haemolytic–uraemic syndrome following an episode of bloody diarrhoea in the first case and diarrhoea in the second. The children were unrelated and their only link was attendance at the nursery. Both children were female, aged three years and two years. There was an interval of about two weeks between the two cases. *E coli* O157 VTEC was
isolated from the faeces of the second case and a serum sample from the index case was positive for antibodies to *E. coli* O157 LPS. Based on these results an outbreak control team was formed. A case definition was produced, the nursery inspected, and an epidemiological investigation instigated. An outbreak questionnaire was issued and this revealed a further 13 children and five members of staff admitting to an episode of diarrhoea during the month of October (the period of probable transmission). Control measures were put in place and no further cases occurred. The duration of the outbreak was determined to be 28 days.

### Patients

A questionnaire concerning possible symptoms was sent to parents of nursery children and nursery staff. As the incubation period for infection with *E. coli* O157 may be up to 14 days and the date of onset for the first case was 14 October, symptoms were sought from 1 October. Those who replied that they had had an episode of diarrhoea (defined as two or more loose motions in a day) were requested to complete a more detailed questionnaire. The questionnaire inquiring about diarrhoeal symptoms was also sent to hospital kitchen staff.

Five members of the nursery staff and 15 children were reported to have had an episode of diarrhoea during the relevant time period associated with the two cases of haemolytic–uraemic syndrome. None of the kitchen staff reported suffering from diarrhoea during this time. Fecal sampling was undertaken but the negative direct cultures prompted the outbreak team to consider serological testing of the staff and children.

Serum samples were obtained from 24 children attending the nursery, 15 of whom had diarrhoea; from five of their parents, one of whom had diarrhoea (these were the parents of the two haemolytic–uraemic syndrome cases and of two other children who had particularly severe diarrhoea); from 11 nursery staff, of whom five had diarrhoea; and from 22 kitchen staff, none of whom had diarrhoea. Sera from 40 other hospital staff and 24 sera from hospital outpatients unrelated to the outbreak were used to assess whether the infection had been more widespread in the hospital. These samples had been collected from hospital staff and patients for other laboratory tests during the outbreak period.

### Methods

#### Bacteriology

Forty one faecal samples from children and nursery staff, irrespective of symptoms, were examined by routine bacterial culture for species of salmonella, shigella, campylobacter, and strains of *E. coli* O157. Faecal specimens were cultured for *E. coli* O157 on sorbitol MacConkey agar (SMAC), and incubated at 37°C for 24 hours (Oxoid, Unipath, Basingstoke, Hampshire, UK). Non-sorbitol fermenting colonies were selected and tested by slide agglutination using antibodies to *E. coli* O157 conjugated with latex particles (Oxoid O157 latex test, Unipath). Colonies agglutinated by the latex reagent were subcultured and identified as *E. coli* using the AP120E test (BioMerieux UK, Basingstoke, Hampshire, UK) and confirmed as belonging to serogroup O157 by slide agglutination with an antisera prepared to *E. coli* O157 (Laboratory of Microbial Reagents, Central Public Health Laboratory, Colindale, UK). Presumptive *E. coli* O157 isolates were serotyped, phage typed, and examined for genes encoding verocytotoxin16–20 by the Laboratory of Enteric Pathogens, Central Public Health Laboratory, Colindale.

#### Serology

One hundred and twenty six sera were examined for antibodies to the LPS of *E. coli* O157 by enzyme linked immunosorbent assay (ELISA) and immunoblotting procedures.21–24 ELISA plates were coated with 0.6 µg LPS and reacted with serum diluted 1/1000 in phosphate buffered saline containing 0.5% Tween-20. Antigen–antibody complexes were detected using alkaline phosphate conjugated goat antiserum to total human immunoglobulin (Sigma, Poole, Dorset, UK) and p-nitrophenol phosphate (1 mg/ml, Sigma) in diethanolamine buffer. The intensity of the final yellow colour was read at 405 nm.

### Results

#### Bacteriology

From 41 faecal samples, *E. coli* O157:H7 belonging to phage type 49 and reacting with a gene probe to VT2 was isolated from one of the cases of haemolytic–uraemic syndrome. The remaining 40 faecal specimens were all culture negative for *E. coli* O157.

#### Serology

Twenty nine of the 126 sera (23%) contained antibodies to the LPS of *E. coli* O157 (table 1). Ten were from children, nine of whom had diarrhoea. Three were from nursery staff, all of whom had diarrhoea, and 11 were from hospital kitchen staff, none of whom had diarrhoea. All five sera from the children’s parents contained antibodies to the LPS of *E. coli* O157; only one parent had reported diarrhoea. The remaining 64 sera from other hospital staff and outpatients—none of whom had diarrhoea—were all negative when tested for antibodies to the LPS of *E. coli* O157.

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**Table 1** Results of screening for serum antibodies to the lipopolysaccharide of *E. coli* O157

<table>
<thead>
<tr>
<th>Category</th>
<th>At risk</th>
<th>Number tested</th>
<th>Seropositive</th>
<th>Seronegative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diarrhoea</td>
<td>Seropositive</td>
<td>Seronegative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No diarrhoea</td>
<td>Seropositive</td>
<td>Seronegative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>31</td>
<td>24</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Nursery staff</td>
<td>11</td>
<td>11</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Kitchen staff</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Parents</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other hospital staff</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Outpatients</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>TOTAL</td>
<td>126</td>
<td>13</td>
<td>8</td>
<td>89</td>
</tr>
</tbody>
</table>
Discussion

E. coli O157 was isolated from the faeces of one of the children with haemolytic-uraemic syndrome attending the hospital nursery; therefore by bacteriology alone evidence of infection would have been detected in only one patient. The failure to isolate E. coli O157 from the remaining children and staff of the nursery who were seropositive could be explained by the time delay between the two cases of haemolytic-uraemic syndrome and the recognition and investigation of an outbreak. Alternatively, those infected were no longer shedding faecal E. coli O157, or the numbers of bacteria excreted were too low to detect using SMAC. Possibly, the use of a more sensitive medium, for example cefixime–tellurite sorbitol MacConkey agar (CTSMAC) or enrichment culture, might have increased the sensitivity of detection.

The negative results from the random testing of 40 sera obtained from hospital staff not connected with the nursery or kitchens and from 24 outpatients, and the absence of reports from the hospital of other cases of diarrhoea among hospital staff who ate hospital canteen food during this period, indicate that this outbreak was limited to the nursery. It is difficult to explain the large number of kitchen staff who were asymptomatic yet antibody positive. Such a high rate of seropositivity could be explained by the kitchen staff having previously been infected following handling of foodstuffs commonly associated with E. coli O157. The kitchen staff may well represent a distinct population within the hospital community and these results merit further study and investigation.

In relation to the outbreak investigation, a common source of infection or possible vehicle of infection was not recognised. The outbreak curve (fig 1) was, however, suggestive of person to person spread. The mildness of the diarrhoal illness among the staff and nursery children who did not have haemolytic-uraemic syndrome shows that not all cases of infection associated with E. coli O157 have prodromal symptoms of haemorrhagic colitis. The investigation highlighted problems that can occur in nurseries where cases of mild diarrhoea may not be notified by parents to nursery staff, and outbreaks may be missed or not recognised in time to allow early investigation and control. The nursery did operate an exclusion policy, but diarrhoeal symptoms occurring in children while away from the nursery would not be reported to the staff, preventing the minimal exclusion recommended by PHLS guidelines for diarrhoeal disease and the more stringent exclusion of two negative stools for cases of E. coli O157. As part of the outbreak investigation a telephone survey of nurseries randomly selected from the local telephone directory showed that although some form of exclusion policy existed no reference was made to recent symptoms occurring while away from the nursery, for example at weekends (Syed Q, unpublished data). This investigation proved the value of a serological test for obtaining evidence of infection with E. coli O157, and its potential for retrospective studies including those in which outbreak investigations are delayed.