New perspectives on the role of *Escherichia coli* O157:H7 and other enterohaemorrhagic *E. coli* serotypes in human disease

P. N. GOLDWATER and K. A. BETTELHEIM*

Microbiology and Infectious Diseases Department, Women’s and Children’s Hospital, North Adelaide, South Australia 5006 and *National Escherichia coli Reference Laboratory, Victorian Infectious Diseases Reference Laboratory, Fairfield, Victoria, Australia

This review compares the rates of detection of non-O157:H7 enterohaemorrhagic *Escherichia coli* (EHEC) with EHEC O157:H7 in outbreaks and sporadic cases of human disease by analysing Australian data and the world literature. Numerous outbreaks of disease have been attributed to EHEC O157:H7. In many studies, isolation rates of this organism have been low and attempts to seek other EHEC have not been made. Ease of isolation and identification of the O157:H7 serotype may have given the impression that this serotype was the sole organism responsible for the outbreaks. Careful review and analysis shows that serotypes other than O157:H7 also play an important role in human disease. Evidence is presented from several overseas outbreaks described in the literature, as well as from investigations of the Adelaide O111:H- outbreak, that suggests an association between severity of disease and multiple infecting serotypes. While not diminishing the role of the O157:H7/H- clone, this review indicates that other serotypes can be responsible for outbreaks as well as cases of sporadic human disease. The current focus on O157:H7 has major implications in terms of diagnosis, the food industry and human health.

Introduction

From the first description 15 years ago [1] of outbreaks of disease caused by enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7, this serotype has tended to dominate the world literature on EHEC. Evidence suggests that EHEC O157:H7 isolates are derived from one particularly successful clone of *E. coli* that has spread around the world. Long before the emergence of this strain, and ever since, there have been reports of human cases and outbreaks of disease caused by serotypes of *E. coli* other than O157:H7. On occasion, these may occur concomitantly with O157:H7 cases, which may lead to the false labelling of an outbreak as one caused by this serotype only.

Investigation of the Adelaide haemolytic uraemic syndrome (HUS) outbreak has provided several insights into the epidemiology of EHEC. Polymerase chain reaction (PCR) detection of *E. coli* stx genes had been introduced as part of research into sudden infant death syndrome. It was fortuitous that the PCR assay was available at the time of the epidemic as it allowed detection of EHEC O111:H-. The hospital laboratory had not introduced selective media for the isolation of *E. coli* O157:H7, but such an approach, without PCR detection of stx genes, would have failed to detect *E. coli* O111:H-. The examination in a different laboratory of a faecal specimen from one of the Adelaide HUS cases who was hospitalised interstate, and the detection in that specimen of a number of EHEC serogroups including O111 and O157, alerted the epidemic investigation to the simultaneous presence of the O157 clone, which was found subsequently in two other patients [2]. Examination of reports of outbreaks of EHEC-related disease indicates that most have been under-investigated from the point of view of the possible involvement of multiple serotypes of *E. coli*. This review provides a fresh look at the epidemiology of *E. coli* and raises issues important to our understanding of why some epidemics are mild clinically and others severe. In addition, the review illustrates some of the important issues involved in the microbiology of food-borne *E. coli* disease.
Isolation rates and outbreaks of disease caused by O157:H7 and other EHEC

A Medline review of the literature was performed to ascertain the detection rate of E. coli O157:H7 compared with other EHEC serotypes in outbreaks of bloody diarrhoea (BD) associated with HUS. The published data from the reported outbreaks of EHEC disease were then analysed to determine the rate at which O157:H7 and non-O157 serotypes were detected. Table 1 shows the isolation rate of EHEC O157:H7 in published studies of outbreaks attributed to this serotype. Specific comment in regard to the manner of investigation, conclusions drawn in regard to the findings and our re-interpretation of the data of many of these cited studies is provided below. Table 2 provides data on seven documented outbreaks attributed to EHEC serogroup O111. Isolates from the Adelaide O111:H− epidemic [2,32] included EHECs other than O111:H−, and included isolates of O157:H7 or O157:H− from three patients. Two of these patients were reported separately [33] and also in the State Coroner's Report. Other Shiga-toxin-producing E. coli (STEC) serotypes including O111:H7 [32] (subsequently shown to be serotype O111:H8; personal unpublished results) were isolated from patients, and STEC serogroups O113, O82:H8, O91, O98, O159 and O157 were isolated, in addition to serotype O111:H−, from the incriminated food source of mettwurst fermented sausage [32].

Data published previously [34,35] on serotypes causing disease in Australia show a wide spectrum of serotypes, including a number that had not been found elsewhere at the time. Many have since been described in other parts of the world. Table 3 shows data attributed to non-O157 and non-O111 EHEC outbreaks and sporadic cases of disease. Included in this table are outbreaks of disease attributed to enteropathogenic E. coli (EPEC) O111:B4 in the middle of this century [30,31]. The clinical features described in the 1953 USA outbreak [31] (diarrhoea, bloody stools, renal failure and anuria, skin petechiae, seizures, ileus, gut infarcts, shock, coma and death) are suggestive of HUS and indicate that these isolates could have carried stx genes. A proportion of classical EPEC strains have been shown on retrospective analysis to carry such genes [46].

**Table 1. Outbreaks of disease attributed to EHEC O157:H7 and its rate of isolation**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>USA</td>
</tr>
<tr>
<td>4</td>
<td>Germany</td>
</tr>
<tr>
<td>5</td>
<td>UK</td>
</tr>
<tr>
<td>6</td>
<td>Japan</td>
</tr>
<tr>
<td>7</td>
<td>UK</td>
</tr>
<tr>
<td>8</td>
<td>USA</td>
</tr>
<tr>
<td>9</td>
<td>USA</td>
</tr>
<tr>
<td>10</td>
<td>Canada</td>
</tr>
<tr>
<td>11</td>
<td>USA</td>
</tr>
<tr>
<td>12</td>
<td>Canada</td>
</tr>
<tr>
<td>13</td>
<td>USA</td>
</tr>
<tr>
<td>14</td>
<td>USA</td>
</tr>
<tr>
<td>15</td>
<td>USA</td>
</tr>
<tr>
<td>16</td>
<td>USA</td>
</tr>
<tr>
<td>17</td>
<td>USA</td>
</tr>
<tr>
<td>18</td>
<td>Australia</td>
</tr>
<tr>
<td>19</td>
<td>Czech Rep</td>
</tr>
<tr>
<td>20</td>
<td>USA</td>
</tr>
<tr>
<td>21</td>
<td>UK</td>
</tr>
<tr>
<td>22</td>
<td>USA</td>
</tr>
<tr>
<td>23</td>
<td>Germany</td>
</tr>
</tbody>
</table>

HUS, haemolytic uraemic syndrome; BD, bloody diarrhoea.

*Of 108 cases, 66 yielded "pathogenic" E. coli not otherwise defined.

†Serogroups identified were O1, O5, O18 and O26.

**Detailed analysis of outbreak reports**

It is manifest from the numerous reports of outbreaks attributed to EHEC O157:H7 that this serotype can at best account for only 60% of isolates in such outbreaks. The study by Pavia et al. [3] found only 16 cases (10%) with O157:H7 out of a total of 157, yet the epidemic was attributed to O157:H7 as the sole causal EHEC. It is noteworthy that the proportion of cases with serogroup O157 infection detected in that outbreak was similar to that of the Adelaide O111:H− epidemic. Furthermore, most studies appear to have neglected non-O157:H7 EHEC during the investigation of the outbreak. This is illustrated by the remainder of this section which considers the methodology used for the investigation of individual outbreaks.
patients yielded O157 strains. The review by Thomas E. I1 from colonies growing on MacConkey agar plates, With a dot-blot ELISA for detecting toxins SLT-I and

In children, 32 were children at the centre; five further

affected, 32 were children at the centre; five further

in a child care centre in Germany there was an outbreak of diarrhoea and BD

in three waves during the summer [4]. Of 39 persons

in a child care centre in Germany there was an outbreak of diarrhoea and BD

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

In the Utah, USA, outbreak [3] of diarrhoea, BD and

In the Utah, USA, outbreak [3] of diarrhoea, BD and

In the Utah, USA, outbreak [3] of diarrhoea, BD and

In the Utah, USA, outbreak [3] of diarrhoea, BD and

Nevertheless, 13 different non-O157 vero toxicigenic

Table 2. Reported outbreaks of EHEC O111

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Year</th>
<th>Country [ref.]</th>
<th>Setting</th>
<th>Number of cases in outbreak*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>O111:H-</td>
<td>1991</td>
<td>Japan [26]</td>
<td>Primary school</td>
<td>234</td>
<td>Unknown</td>
</tr>
<tr>
<td>O111:H-</td>
<td>1992</td>
<td>Italy [27]</td>
<td>Community</td>
<td>9</td>
<td>Unknown</td>
</tr>
<tr>
<td>O111:H-</td>
<td>1992</td>
<td>France [28]</td>
<td>Primary school</td>
<td>26</td>
<td>Unknown</td>
</tr>
<tr>
<td>O111:H-</td>
<td>1996</td>
<td>USA [29]</td>
<td>Family</td>
<td>5</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Possible EHEC O111 outbreaks:


*Sporadic cases of HC or HUS associated with O111:H- have also been reported from Belgium, Germany, Austria and the Czech Republic.

19 follow-up and 24 contacts (total 690), a maximum

An outbreak in a nursery school in Japan in late

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

In the Utah, USA, outbreak [3] of diarrhoea, BD and

In the Utah, USA, outbreak [3] of diarrhoea, BD and

In the Utah, USA, outbreak [3] of diarrhoea, BD and

Nevertheless, 13 different non-O157 vero toxicigenic

Table 3. Outbreaks and sporadic disease caused by non-O157:H7 and non-0111 enterohaemorrhagic E. coli

<table>
<thead>
<tr>
<th>Serotype Year Place Setting</th>
<th>Number of cases Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>O26</td>
<td>1979 UK BD; sporadic/community</td>
<td>3 Unknown</td>
</tr>
<tr>
<td>O26:H-</td>
<td>1989-91 UK HC/HUS</td>
<td>...</td>
</tr>
<tr>
<td>O26:H11</td>
<td>1987-90 Germany</td>
<td>...</td>
</tr>
<tr>
<td>O26:H11</td>
<td>1984-93 Japan</td>
<td>Sporadic/intrafamilial</td>
</tr>
<tr>
<td>O26:H11</td>
<td>1978-79 New Zealand Diarrhoea</td>
<td>...</td>
</tr>
<tr>
<td>O26:H11</td>
<td>1992 Australia HUS</td>
<td>1 Unknown</td>
</tr>
<tr>
<td>O26:H11</td>
<td>1988-95 Czech Rep. HUS</td>
<td>5 Unknown</td>
</tr>
<tr>
<td>O91:H21</td>
<td>1987-90 Germany HUS</td>
<td>...</td>
</tr>
<tr>
<td>O103:H2</td>
<td>1987-89 France, HUS</td>
<td>6 Unknown</td>
</tr>
<tr>
<td>O103:H2</td>
<td>USA Urinary infection</td>
<td>1 Unknown</td>
</tr>
<tr>
<td>O104:H21</td>
<td>1994 USA (Montana) Outbreak/gastroenteritis</td>
<td>17 Unknown</td>
</tr>
<tr>
<td>O113:H21</td>
<td>1997 Australia BD TTP</td>
<td>1 Unknown</td>
</tr>
<tr>
<td>O145:H</td>
<td>1984 Japan Diarrhoea</td>
<td>100 Unknown</td>
</tr>
<tr>
<td>Omt:H2</td>
<td>1991 Japan School</td>
<td>89 Unknown</td>
</tr>
<tr>
<td>Omt:H19</td>
<td>1984-93 Japan</td>
<td>?</td>
</tr>
<tr>
<td>O165</td>
<td>1995 Japan</td>
<td>?</td>
</tr>
</tbody>
</table>

BD, bloody diarrhoea; TTP, thrombotic thrombocytopenic purpura; HUS, haemolytic uraemic syndrome.

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

sorbitol-MacConkey agar culture (SMAC) was used to

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retarded,

In the Utah, USA, outbreak [3] of diarrhoea, BD and

HUS in two institutions for the mentally retard
SMAC was used to detect VTEC. There were 31 cases of BD of which 14 (45.2%) yielded VTEC O157:H7 and 23 cases of diarrhoea in which one (4.3%) yielded VTEC O157:H7. While meat patties were incriminated on epidemiological grounds, VTEC O157:H7 could not be isolated from uneaten patties. Again, only SMAC was used for detection of NSF colonies.

An outbreak in a small town in Missouri from Dec. 1989 to Jan. 1990, in which 243 people were affected, was described by Swerdlow et al. [9]. In total, 86 patients had BD and the remaining 157 had diarrhoea. SMAC was used to isolate EHEC O157:H7, which was found in 18 (76%) of 25 patients with BD and from two (28.6%) of seven patients with diarrhoea. Although the water supply was incriminated on epidemiological evidence, no VTEC was isolated from the water. Another outbreak involving six Canadian Arctic communities occurred between June and Oct. 1991 [10] in which 521 individuals developed diarrhoea, including 103 with BD. Although SMAC was used to isolate VTEC, verotoxin (VT) was looked for in polymyxin extracts of colony sweeps and in faecal extracts, and 129 patients (24.8%) were confirmed by SMAC was used for detection of NSF colonies.

The June 1995 Colorado child care outbreak [16] involving 141 children, including 24 cases of diarrhoea and BD, yielded E. coli O157:H7 from 12 cases (50%). The authors failed to describe the method of isolation, but as no other E. coli was mentioned, it is assumed that an O157-specific technique was used. One reason frequently given to explain the lack of isolation of E. coli O157:H7 is the very rapid clearing of the organism from the gut and the fact that isolation of the organism is unlikely after the first week of symptoms. In this outbreak it was noted that the duration of shedding had a range of 11–57 days with a mean of 29 days.

In the Sept. 1993 Connecticut, USA, outbreak among picnic attendees [17], 23 persons met the case definition, of whom 10 had stools cultured with five (50%) positive for E. coli O157:H7. All positive individuals had BD. Incriminated meat patties also yielded E. coli O157:H7. It is interesting to note that the laboratory had begun screening for E. coli O157 only in June of that year.

The outbreak in March 1996 in south Queensland, Australia [18] was labelled as having been caused by E. coli O157 on the basis of SMAC. Disease was mild in this outbreak and 57 people provided 84 faecal specimens. Six people were infected with O157, including three patients, an asymptomatic relative and two food handlers. Twenty-one other associated people, including two patients and six family contacts, were negative for O157, as were 30 people used as controls. Thus O157 was isolated from three (60%) of five patients or, overall, from six (22.2%) of 27 patients, contacts and suspect food handlers.

In an outbreak in northern Bohemia in 1988 [19], there were five cases of HUS and one case of HC. Two of the HUS cases and the HC case yielded O157:H7, while two others yielded EHEC O26 and one case yielded STEC O1. One of the HUS cases with O157 also had STEC O5. SMAC was used in conjunction with specific tests to select non-O157 STEC.

In the June–Nov. 1995 New York hospital study [20], 270 stool specimens described as having the following characteristics: ‘liquid, semiliquid, mucous, blood, ...’ were tested with SMAC and the Meridian EHEC test kit. Of the 270 specimens, 11 were positive for SLT and six (54.5%) of these were positive for O157:H7. Other serotypes isolated were O88:H–, O103:H–,
been misrepresented. For instance, clinical severity in been attributed to EHEC 0157, which was found in clinical parameters associated with EHEC may have relation to apparent single agent al.

EHEC 0157:H7, then it is plausible that certain mentioned outbreaks, would almost certainly have three patients [33]. It follows that if the majority of that other serotypes are not sought.

The christening party outbreak of diarrhoea and HC affecting 26 of 93 people and resulting in one case of HUS [21] was investigated by SMAC. Faeces from 23 cases and 33 asymptomatic guests were cultured on SMAC and colonies tested for the stx1 and stx2 genes by PCR. Of 23 cases examined, 13 (56.5%) were O157 positive and three (9.1%) of 33 asymptomatic guests were O157 positive. It is not clear from the description of the methods which colonies were tested by PCR, but if only sorbitol-negative colonies were tested then this would limit the possibilities for non-O157 EHEC isolation.

The investigation of several outbreaks has also been biased by limiting the case definition to positive O157:H7 culture or serology. The outbreak of diarrhoea, HC and HUS associated with bathing in a lake in Illinois, USA, serves to illustrate this point [22]. In the investigation, 12 cases were identified, of which seven (58.3%) were positive for EHEC O157:H7 by culture, yet the culture method was not disclosed. Case definition was based on culture of EHEC O157:H7 or a positive serological reaction for the O157 serogroup, or both. Therefore, it would seem that other serotypes were not sought.

Returning to the Adelaide HUS epidemic of summer 1995, had there been no system available for detecting O111 then this outbreak, like most of the above-mentioned outbreaks, would almost certainly have been attributed to EHEC O157, which was found in three patients [33]. It follows that if the majority of outbreaks have had their investigations limited to EHEC O157:H7, then it is plausible that certain clinical parameters associated with EHEC may have been misrepresented. For instance, clinical severity in relation to apparent single agent versus mixed EHEC infection may be important. The study by Rodrigue et al. [11] drew attention to the benign clinical course. Could this and other clinically mild outbreaks [18] have been caused by EHEC O157:H7 alone? That is, a 'single agent' outbreak accounting for the benign clinical features. On the other hand, could severe outbreaks, with relatively large numbers of cases of HUS, such as occurred in the Adelaide epidemic, be the result of multiple strains acting 'in concert'? Our serological analysis of the Adelaide outbreak has shown significant antibody responses to a much wider range of E. coli serotypes than was found in the patients or the incriminated food source of fermented sausage (mettwurst) (personal unpublished observations). Until such time when outbreaks are investigated for all possible EHEC, the answer to the above question with regard to possible clinical correlations between severity of disease and numbers of infecting serotypes (and hence, possibly, Shiga toxin types) will remain unanswered. Furthermore, this work has been confounded by the absence of an animal model that accurately reflects the mechanisms of colonisation and pathogenesis of disease in man. Recently, Pierard et al. [47] screened over 17,000 faeces submitted for routine culture for VTEC and showed that cases of HUS were observed only in association with EHEC O157:H7. Seventeen of 21 Adelaide epidemic HUS patients had antibody to serogroup O157, while this serogroup was found in only two of these patients' faeces (personal unpublished observations). Therefore, these serological data support the pathogenic role of EHEC O157; however, on the other hand, there was no difference between the number of patients with antibody to this serogroup who had complications of HUS (chronic renal failure, infarcts, etc.) and the number of O157 seropositive patients without complications. Therefore, the role of O157 infection in disease severity needs further study, as does the number of infecting EHEC serotypes per patient.

Conclusions

While not diminishing the role of the O157:H7 clone, this review illustrates the importance of recognising that other serotypes are responsible for outbreaks as well as cases of sporadic human disease. It is also apparent from the literature that a wide variety of EHEC impinge on the human host from a wide range of food and non-food sources. Failure to identify the source is not uncommon. On the basis of this review, it is clear that non-O157:H7 are important (and probably underestimated) causes of disease. Clearly, other EHEC will be missed unless they are looked for specifically with a 'broad brush' approach (e.g., PCR detection of stx genes [32] or direct detection of toxins [48] in faeces or faecal cultures). Medical diagnostic and public health laboratories should be encouraged to use techniques that detect stx genes or toxins in clinically and epidemiologically appropriate specimens, and should not restrict themselves to looking for O157:H7. The current focus on EHEC O157:H7, and seeming neglect of other EHEC, has major implications in terms of diagnosis, the food industry and human health. In addition, the practice of some authors of implying that EHEC O157:H7 is part of the definition of HUS should be discouraged. Ignoring the well-
established data presented in this review that EHEC other than O157:H7 also cause HUS is clearly established, data presented in this review that EHEC prevention and control of EHEC infections held in gone some way toward recognizing the problem of this restrictive approach to diagnosis of EHEC-related diseases. On the same basis, the food industry should also be encouraged to follow this line by being cognisant with the importance of non-O157:H7 EHEC as well as O157:H7.

References


45. Uchida H, Kanegane H, Yoshiya K et al. [Four cases of hemolytic uremic syndrome (HUS) associated with serotype O165 verotoxin producing *Escherichia coli* (VTEC) identified by LPS-solid phase enzyme-linked immunosorbent assay (ELISA)]. *Kansenshogaku Zasshi* 1995; 69: 678–683.


