

## Section 4: Clinical aspects of VTEC infections

Patients infected with VTEC O157 produce serum and salivary antibodies to O157 lipopolysaccharide antigens.

During infection with VTEC, inflammatory mediators such as  $\text{TNF}\alpha$  and IL-1 are produced by cells of the immune system. These play a role in the pathogenesis of human infections by increasing the number of cell-surface receptors for VT.

### 4.1 Introduction

In this section the clinical aspects of infection with Verocytotoxin-producing *Escherichia coli* (VTEC) in man are reviewed. The clinical spectrum of infection with VTEC ranges from mild diarrhoea through to haemolytic uraemic syndrome (HUS) – a common cause of acute renal failure in children. Treatment options are limited although several promising developments have emerged in recent years. These are reviewed at the end of the section.

### 4.2 Clinical syndromes associated with human Verocytotoxin-producing *Escherichia coli* infection

#### 4.2.1 Susceptibility

Susceptibility to infection with VTEC appears to be general, i.e. the population as a whole is potentially vulnerable to becoming infected. Those at the extremes of age are considered to be at greatest risk.

#### 4.2.2 Incubation period and infectious dose

The incubation period for VTEC O157 infection is usually around three to four days (Mead and Griffin 1998; Besser *et al.* 1999). However, longer incubation periods have been recorded (Table 4.1).

**Table 4.1 Documented incubation periods for VTEC infections**

Year of outbreak	Mode of transmission	Incubation period	Comments	Reference
1985	Foodborne followed by person-to person transmission	4 to 9 days	Population affected predominantly elderly. Vehicle = contaminated sandwiches	Carter <i>et al.</i> , (1987)
1990	Foodborne	1 to 14 days	Community outbreak associated with a restaurant.	Marsh <i>et al.</i> , (1992)

It is generally agreed that the infectious dose for VTEC O157 infection is low (Griffin and Tauxe 1991; Willshaw *et al.* 1994; Bolton and Aird 1999). Table 4.2 summarises the data available from key outbreak investigations that give insight into the infectious dose for humans.

**Table 4.2 Summary of data from key outbreak investigations showing evidence of infectious dose**

Year of outbreak	Mode of transmission	Estimated infectious dose	Comments	Reference
1992/3	Foodborne	< 700 organisms	Vehicle = ground beef patties. Median most probable number of organisms = 1.5 per gram (range, < 0.3-15) or 67.5 organisms per patty (range, < 13.5-675).	Tuttle <i>et al.</i> , (1999)
1996	Foodborne	<50 organisms	Vehicle = dry salami	Tilden <i>et al.</i> , (1996)
1996	Foodborne	31.0 cfu* (children) 35.0 cfu* (adults)	Vehicle = contaminated school lunches (Morioka City, Japan)	Teunis <i>et al.</i> , (2004)
2000	Environmental	4 – 24 organisms	Close fit with the Crockett model ( <i>Shigella</i> dose-response model)	Strachan <i>et al.</i> , (2001)

\*cfu = colony-forming unit

The incubation period and infectious dose for non-O157 VTEC appear to be similar to those of VTEC O157.

### **4.2.3 Acute infection**

The clinical manifestations of infection with VTEC O157 range from asymptomatic infection (see Section 4.3.1) through mild diarrhoea to haemorrhagic colitis (HC) and the severe complications of HUS and thrombotic thrombocytopenic purpura (TTP) and even death.

#### **4.2.3.1 Diarrhoeal disease**

Following an incubation period of around three to four days, patients normally develop watery diarrhoea, from which between 25% and 75% recover, having suffered only mild illness (Besser *et al.* 1999). However, Spacek *et al.* (2004) suggested that VTEC O157 infection was the cause of chronic diarrhoea in two of their patients.

### **4.2.4 Complications**

#### **4.2.4.1 Haemorrhagic colitis (HC)**

For those who develop HC, bloody diarrhoea typically starts on the second or third day of illness and the amount of blood varies from stools streaked with blood to stools that contain only blood. Typically, this is in the absence of a high fever. In a recently published cohort study in Denmark (Ethelberg *et al.* 2004), just over one third of people infected with *E. coli* O157 developed bloody diarrhoea. When bloody diarrhoea is severe, the clinical presentation can be confused with intussusception in children and, in the elderly, with inflammatory bowel disease or ischaemic colitis. There is mounting evidence that VTEC O157 more frequently cause bloody diarrhoea than non-O157 VTEC (Jelacic *et al.* 2003; Ethelberg *et al.* 2004; Werber *et al.* 2004). In the study by Ethelberg *et al.* (2004), the two most frequently occurring O groups were O157 and O103 and these were each associated with development of bloody diarrhoea, and also the presence of the *eae* and *vtx2* genes. Recovery from illness usually occurs over approximately one week in the majority of patients and there are no obvious long-lasting effects. However, between 5-20% of people infected with *E. coli* O157: H7 will go on to develop HUS or TTP (Tarr *et al.* 1990; Boyce *et al.* 1995; Klein *et al.* 2002; Locking *et al.* 2004).

#### 4.2.4.2 Haemolytic uraemic syndrome

One of the most serious manifestations of infection with VTEC in humans is HUS. HUS comprises a triad of microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure (Fitzpatrick 1999). The link between VTEC O157 infection and HUS was first made by Karmali *et al.* (1983). At least 80% of childhood HUS is attributable to infection with VTEC (Banatvala *et al.* 2001), mainly serogroup O157, although other serogroups are implicated (Misselwitz *et al.* 2003). The risk of a child less than 10 years of age developing HUS is approximately 15% (Tarr *et al.* 2005). The peak incidence of HUS is in children under 5 years of age and recently published incidence estimates are presented in Table 4.3.

In Argentina where HUS is endemic, approximately 400 new cases are reported annually by hospital nephrology units. In 2003, the estimated annual rate of HUS in Argentina was 11.5 cases per 100,000 in children under five years of age, with more than 7,000 cases of HUS reported since 1965 (Rivas *et al.* 2003). The genotype *vtx2+vtx2vh-a* (*vtx2d*), which is considered to be highly virulent (Nishikawa *et al.* 2000), is prevalent in VTEC O157 strains in Argentina from human specimens and food samples (Chinen *et al.* 2003). Most of the *E. coli* O157: H7 strains isolated from HUS cases harbour the *eae/vtx2+vtx2vh-a/EHEC-hlyA* genes (Dr. Isabel Chinen, personal communication).

**Table 4.3 Incidence of HUS in children less than five years of age and serotypes of VTEC (microbiologically and/or serologically confirmed) associated with HUS in population-based studies published since 2000**

Years of study	Location (N = number of subjects in the study)	Average annual incidence (per 100,000) in children < 5 yrs)*	Serotype/group identified in VTEC + cases (%)	Reference
1997-2001	UK & Ireland (N = 413)	1.54	O157 (99%) O26 (1%)	Lynn <i>et al.</i> , (2005)
1998-99	New York, USA	2 (D+HUS cases)	O157 (100%) – note tests for non-O157 VTEC not undertaken	Chang <i>et al.</i> , (2004)
1988-2000	Italy (N=342)	0.75	O157 (41%) O26 (19%) O111 (9%) O145 (9%) O103 (5%)	Tozzi <i>et al.</i> , (2003)
1997-2000	Austria & Germany (N=394)	1.71 (Germany) 0.56 (Austria)	O157: H7 (57%) SF O157: H <sup>-</sup> (10%) O26: H11/H <sup>-</sup> (15%) O145: H <sup>-</sup> (9%) O103: H2/H <sup>-</sup> (3%) O111: H <sup>-</sup> (3%) Others (3%)	Gerber <i>et al.</i> , (2002)
1994-1998	Australia (N = 98)	1.35	O111 (62%) O157 (18%) O113: H21 (8%) Others (12%)	Elliott <i>et al.</i> , (2001)
1987-1991	USA (N = 83)	Not stated (55% <5 yrs of age)	O157 (80%) in a sub-set of 55 patients	Banatvala <i>et al.</i> , (2001)
1993-1996	France (N=286)	1.8	O157 (67%)	Decludt <i>et al.</i> , (2000)

\* Direct country comparisons in children over the age of five are more difficult since the cut-off age for paediatric cases varies. D+HUS denotes diarrhoea-associated HUS

The time between onset of *E. coli* O157 diarrhoea and development of HUS is typically between 5 and 13 days (median 7 days) (Tarr *et al.* 2005). Not all VTEC-associated HUS cases present with diarrhoea. Thrombocytopenia is usually the first abnormality to develop in children developing HUS.

Since enteric VTEC infections are seldom accompanied by bacteraemia, systemic complications are thought to arise from lesions caused by circulating Verocytotoxins (VT). Even before HUS develops, prothrombotic coagulation abnormalities can be detected. However, these abnormalities occur in many VTEC O157 infected children, regardless of whether or not they progress to develop HUS (Chandler *et al.* 2002).

It has been postulated that the diarrhoeal phase of VTEC infection is caused by mesenteric ischaemia initiated by circulated VT, rather than through direct injury to the intestinal epithelium by VTEC (Tarr *et al.* 2005). The majority of VTs preferentially bind to glycosphingolipid globotriosylceramide (Gb3) receptors, which are found on renal glomerular endothelial, mesangial and tubular epithelial cells. Binding of VT1 and VT2 to the surface of human neutrophils, both *in vitro* and *in vivo*, has been shown previously (te Loo *et al.* 2001a; te Loo *et al.* 2001b; Tazzari *et al.* 2004; Brigotti *et al.* 2006).

*In vivo*, VT2 has been found bound to polymorphonuclear leukocytes in the peripheral circulation of children with HUS (te Loo *et al.* 2001b). Using flow cytometry it has been possible to detect VT bound to PMNs from patients with HUS (Tazzari *et al.* 2004). A study of HUS patients by Brigotti *et al.* (2006) indicated that VTs bound to circulating PMN remain detectable several days after onset of prodromal diarrhoea. They concluded that immunodetection of VT on neutrophils is therefore a valuable tool for laboratory diagnosis of VTEC infection in HUS. *In vitro* experiments demonstrate that VTs damage monocytes, in turn mediating a thrombotic response (Tarr *et al.* 2005).

On the basis both of the extreme haematological disturbances before and during HUS, and the histopathological appearance of diseased kidneys, the renal damage that occurs during HUS is considered to be thrombotic rather than vasculitic (Tarr *et al.* 2005). Tarr *et al.* (2005) surmised that the development of HUS is related to the degree of prothrombotic activation early in the infection and to the intensity of the subsequent coagulation response.

In recently published individual studies, mortality rates for diarrhoea-associated HUS of 2.5% (Lynn *et al.* 2005), 2.8% (Gianviti *et al.* 2003) and 6% (Banatvala *et al.* 2001) have been reported. For non diarrhoea-associated cases, the mortality tends to be much higher. In an analysis of VTEC O157

associated disease burden in the Netherlands, Havelaar *et al.* (2004) showed that the mean disease burden was 116 Disability Adjusted Life Years (DALYs) per year (90% confidence interval 85-160). Mortality due to HUS (58 DALYs), and end-stage renal disease (ESRD) (21 DALYs) and dialysis due to ESRD (21 DALYs) represented the main determinants of disease burden. In a retrospective, hospital-based case study conducted in Scotland, 6 of the 32 adult patients with VTEC O157 infection developed HUS, 27 were fully recovered by time of hospital discharge, three had impaired renal function and two died in hospital (Cadwgan *et al.* 2002).

Non-renal complications of HUS include neurological problems (irritability, confusion, stroke, seizures and coma) in about 10% of patients, cardiac dysfunction and congestive cardiac failure, pulmonary oedema (secondary to fluid overload), adult respiratory distress syndrome, intestinal perforation and pancreatitis (Tarr *et al.* 2005).

#### **4.2.4.3 Thrombotic thrombocytopenic purpura (TTP)**

Haemolytic uraemic syndrome has hitherto been considered the main complication of VTEC infection in children and TTP a major consequence in adults. The clinical presentation of these two syndromes is very similar but in TTP there are usually also fever and neurological manifestations. At presentation the predominant feature in HUS is renal failure, whereas the neurological symptoms predominate in TTP (Richards *et al.* 2002). However, accumulating evidence suggests that HUS, congenital TTP and idiopathic TTP should no longer be considered to be similar forms of the same disease, despite the similar clinical presentation. They are caused by discrete pathological mechanisms and the previous terminology that linked HUS with TTP is no longer recommended (Tarr *et al.* 2005). TTP is associated with deficient activity of a circulating von Willebrand factor-cleaving metalloprotease, known as ADAMTS13 (Tsai *et al.* 2001), which is only rarely seen in HUS patients (Hunt *et al.* 2001).

#### **4.2.5 Late sequelae**

A high prevalence of renal damage has been reported (Fitzpatrick *et al.* 1991; Siegler *et al.* 1994; Blahova *et al.* 2002) post HUS. In a recent meta-analysis of the long term prognosis for diarrhoea-associated HUS, death or end-stage renal disease (ESRD) occurred in about 12% of patients and 25% of survivors demonstrated long-term renal sequelae (Garg *et al.* 2003). Risk factors for long-term sequelae were severity of the HUS episode and the need for

dialysis (Garg *et al.* 2003). Arterial hypertension has also been noted in long-term follow-up (De Petris *et al.* 2004).

### 4.3 Human VTEC Infections

#### 4.3.1 Asymptomatic infection

Evidence for asymptomatic carriage of VTEC in humans comes from community studies and follow-up of cases of HUS. In a community study of infectious intestinal disease in England, 27 VTEC strains were isolated, 15 of which were isolated from asymptomatic controls (Evans *et al.* 2002). Although the sample size was very small, there was a suggestion that asymptomatic carriage of non-O157 VTEC might be higher in adults than in children. In population-based surveillance in Wales, nearly 15% of all laboratory-confirmed VTEC O157 infection arose in asymptomatic individuals (Chalmers *et al.* 1999). In Scotland in 2003, around 6.5% of infections occurred in people with no symptoms (Locking *et al.* 2004).

In a retrospective study of children with diarrhoea-associated HUS, asymptomatic carriage of VTEC O157 was a frequent finding amongst other members of the household (Heuvelink *et al.* 1999). More recently, Verocytotoxin has been demonstrated bound to circulating polymorphonuclear leukocytes in approximately 80% of household contacts of HUS cases (te Loo *et al.* 2001a), suggesting that the extent of spread in case households might be much greater than previously thought.

Ludwig *et al.* (2002c) investigated the presence of immunoglobulin (Ig) G antibodies to VT2 and VT1 in household contacts of HUS cases. Using a combination of isolation and molecular and serological techniques, they found that 26% (25 of 95) of household contacts had evidence of VTEC infection, of whom 18 (72%) were asymptomatic. Finally, a small proportion of farmworkers surveyed in Italy, all of whom displayed no symptoms, but were an asymptomatic household contact, were found to be stool culture positive for VTEC O157 (Silvestro *et al.* 2004).

On farms, VTEC can be dispersed amongst domestic as well as wild animals and the environment. In Norway, the endemic level of *E. coli* O157: H7 in sheep and cattle is reported to be of a low level or prevalence compared with many other European countries (Vold *et al.* 1998; Urdahl *et al.* 2001). Despite this, a study of a Norwegian farm by Wasteson *et al.* (2005) revealed that *E. coli* O157: H7 can occur and have a widespread distribution yet still not appear to result in any detectable increase in gastrointestinal disease in the

associated population. However, it was not possible to draw firm conclusions from this data set, which was acknowledged as being sparse. Furthermore, the authors commented that the observed absence of human disease could also be attributed to the lack of cytotoxic activity of the strain isolated in this study (Wasteson *et al.* 2005).

Frequent exposure to VTEC on farms resulting in acquired immunity among farm workers to certain VTEC serotypes is not fully understood.

Asymptomatic carriage, albeit for short periods, of VTEC by dairy farm families has been reported previously (Rahn *et al.* 1997). In a study by Silvestro *et al.* (2004), 1.1% of farm workers in Northern Italy were reported to be positive for VTEC O157, and this pathogen was also isolated from a house-hold contact, yet everyone remained asymptomatic. The finding that VTEC, including VTEC O157 can be found in farm workers and other people living and working in rural communities has not been fully explored. The extent of this apparent acquired immunity would be of interest if a human vaccine was ever to be developed. Knowledge of the VTEC serotypes associated with asymptomatic carriage in man and the virulence profiles of these strains would also help to improve our knowledge of any natural associations that can occur between VTEC and humans in rural communities.

#### **4.3.2 Relationships between severity of infection and pathogenicity-associated risk factors, and other risk factors**

Diarrhoea-associated (D+) HUS is mostly secondary to infection with VTEC O157, although other serogroups have been implicated (see below). Strains of VTEC that produce both VT1 and VT2 appear to be less virulent than those producing VT2 but not VT1 (Nataro and Kaper 1998). Verocytotoxin 1 is highly conserved, whilst VT2 shows sequence variation (Nataro and Kaper 1998). Friedrich *et al.* (2002) have suggested that the presence of VT(2d) non activatable or VT(2e) may predict milder disease with minimal risk of HUS.

VTEC strains also frequently harbour the locus of enterocyte effacement (LEE) pathogenicity island, which, amongst other things, encodes the adhesin intimin. Several other factors may be involved in the pathogenic process; among them enterohaemolysin is also produced by many VTEC strains (Nataro and Kaper 1998). In a study in Finland, a particular virulence profile, namely O157: H7: phage type (PT) 2: Stx(2): Stx(2c): *eae* gene (intimin encoding) positive: Ehly (enterohaemolysin) positive, was significantly associated with HUS and bloody diarrhoea compared with other strain profiles (Eklund *et al.* 2002). In the United Kingdom and Republic of Ireland, the risk

of developing diarrhoea-associated HUS was significantly higher in children infected with VTEC O157 PT 2 and PT 21/28 compared with other PTs (Lynn *et al.* 2005). In a cohort of Danish VTEC positive patients, Ethelberg *et al.* (2004) found that risk factors for bloody diarrhoea were the *eae* and *Stx2* genes, O groups O157 and O103, and increasing age. Risk factors for HUS in the same cohort were presence of the *vtx2* (*stx2*) and *eae* genes, being a child, and having bloody diarrhoea. Co-infection with more than one VTEC strain has also been suggested to affect outcome. In following up patients from a large HUS outbreak in Australia, Kulkarni *et al.* (2002) found that patients with severe renal failure tended to develop antibodies to a larger number of serogroups than those with moderate or mild renal impairment. There is also evidence from studies by Gamage *et al.* (2003) that the susceptibility of intestinal flora to attack by phage could contribute to the severity of infection. Whereas the normal human flora appear to be resistant to phage attack, it was discovered that 10% of intestinal *E. coli* were able to amplify toxin production and therefore it is conceivable that these bacteria could contribute to progression of severe disease in human VTEC infections (Gamage *et al.* 2003) (see Section 2.5.2).

In a comparison of 940 non-O157 VTEC strains isolated from humans with sporadic illness in the US between 1983 and 2002 by Brooks *et al.* (2005), *stx2* (*vtx2*) was reported to be strongly associated with HUS, which supports previous findings in the UK (Jenkins *et al.* 2003). In the US study, *eae* was reported to be strongly associated with an increased risk of bloody diarrhoea (Brooks *et al.* 2005).

#### **4.3.3 Associations between serogroups and human disease**

The association between virulence types and human disease is described in Section 3.9.2. Although VTEC O157 is the predominant pathogen in man, there are more than 150 non-O157 serotypes, with O26, O103, O111, O121, and O145 being the serotypes most commonly associated with human disease (Bettelheim 2003). Table 4.3 shows that, whilst VTEC O157 is the predominant pathogen causing HUS in man, other serotypes have also been implicated.

In the study by Brooks *et al.* (2005), the most common non-O157 VTEC serotypes isolated from humans with sporadic illness were O26 (22%), O111 (16%), O103 (12%), O121 (8%), O45 (7%) and O145 (5%). In this study, VTEC O111 was the only serogroup statistically associated with HUS and was reported to be the second most common bacterial cause of this syndrome after VTEC O157: H7.

#### 4.3.4 Host factors

The humoral antibody response of patients to infection with VTEC has been investigated but has focussed primarily on antibody production to VT, and the lipopolysaccharide antigens expressed by the respective VTEC serogroup. The observation that sera from patients with HUS were able to neutralise VT (Karmali *et al.* 1983) suggested that patients infected with VTEC may mount an antibody response to VT during infection. Similar studies supported this theory (Rowe *et al.* 1993; Yamada *et al.* 1994); however, the role of VT-specific antibodies in the observed neutralisation was cast into doubt when neutralising ability was detected in sera from healthy controls (Barrett *et al.* 1991; Bitzan *et al.* 1993). The neutralising ability of serum was shown to probably not involve VT-specific antibodies when it was demonstrated that VT binds to amyloid protein P in serum (Kimura *et al.* 2001; Marcato *et al.* 2003), a phenomenon which would prevent the action of VT on Vero cells in traditional neutralisation assays, giving the false impression that anti-VT antibodies were involved.

To examine the sera from patients with HUS for antibodies to VT, the techniques of ELISA and immunoblotting were used in association with purified VT1 and VT2 (Chart *et al.* 1993b), but VT-specific antibodies were not detected, suggesting that patients probably do not express antibodies to VT during infection. A study examining the incidence of antibodies to VT in apparently healthy members of rural communities (Evans *et al.* 2000) showed that people with occupational exposure to cattle may produce antibodies to VT. Studies by Tesh *et al.* (1991) showed that VT bound to human endothelial cells via galactose  $\alpha$  1-4 galactose  $\beta$  1-4 glucose ceramide (Lingwood *et al.* 1987) such that the levels of VT in the circulation might be too low for immune recognition. The levels of protein toxin would probably be exceedingly small and probably insufficient to evoke an immune response. Thus it appears that only a minority of patients with VTEC infection have a detectable serum antibody response to the particular VT type involved.

The observation that sera from patients with HUS, caused by *E. coli* O157, contained agglutinating antibodies to *E. coli* O157 (Notenboom *et al.* 1987) suggested that patients might produce antibodies specific for antigens located on the bacterial cell surface. Using the technique of immunoblotting, patients infected with *E. coli* O157: H7 were shown to produce serum antibodies to predominantly the lipopolysaccharide (LPS) of *E. coli* O157 (Chart *et al.* 1989). Subsequent work confirmed this (Barrett *et al.* 1991; Bitzan *et al.* 1993; Karch *et al.* 1996), and the detection of serum antibodies to *E. coli* O157 was developed into a routine diagnostic test (Chart *et al.* 1989; Chart *et*

*al.* 1991). Patients infected with VTEC O157 may also produce salivary antibodies to the O157 LPS antigens (Chart and Jenkins 1998). The production of antibodies to the LPS of VTEC, other than *E. coli* O157, has been demonstrated (Chart and Rowe 1990; Chart *et al.* 1993a).

Tumour necrosis factor (TNF) and Interleukin-1 (IL-1) are inflammatory mediators produced by cells of the immune system, such as macrophages (van Deuren 1994). The lipid A moiety of bacterial LPS, as expressed by VTEC O157, can have a direct effect on the production of these cytokines. This, in turn, has a direct influence on the pathogenesis of certain bacterial infections. The effects of Shiga toxin on human umbilical vein endothelial cells (HUVEC) were examined and it was demonstrated that LPS from *Sh. dysenteriae*-1 increased the cytotoxicity of Shiga toxin for these cells (Tesh *et al.* 1991) although the LPS alone was not cytotoxic for HUVEC cells. It was shown that TNF, in association with Shiga toxin, caused increased cytotoxicity (Louise and Obrig 1991) and it appeared that TNF made HUVEC more susceptible to Shiga toxin (Louise and Obrig 1992).

Studies using radio-labelled VT1 showed that TNF $\alpha$ , IL-1 and LPS increased the binding of VT1 to HUVEC cells (van de Kar *et al.* 1992) and it was suggested that TNF $\alpha$  and IL-1 were responsible for actually increasing the number of Gb3 receptors on the cell surface of HUVEC. In fact, VT was shown to have a direct effect on increasing the activities of galactosyl-transferase and protein kinase C (van de Kar *et al.* 1995), resulting in an increase in the number of cell surface Gb3 toxin receptors, allowing greater numbers of VT molecules to bind to eukaryotic cells expressing these toxin receptors. Cytokine-mediated increases in the expression of eukaryotic cell-surface receptors for VT have been demonstrated *in vitro*, and different cell types increase the number of Gb3 receptors to varying degrees (Tesh 1998). Cytokines clearly do play a role in the pathogenesis of human infections with VTEC O157 by increasing the number of cell-surface toxin receptors.

Major risk factors for acquiring VTEC O157 infection are discussed in Section 5. Proposed risk factors for developing HUS include:-

- Extremes of age (<15 years or >65 years) (Dundas *et al.* 2001)
- Raised white cell count (Bell *et al.* 1997; Ikeda *et al.* 2000; Dundas *et al.* 2001)
- Raised C reactive protein (Ikeda *et al.* 2000)
- Elevated temperature (Ikeda *et al.* 2000)

- Blood group O (Blackwell *et al.* 2002) although in other investigations no association with ABO blood group has been found (Shimazu *et al.* 2000; Jelacic *et al.* 2002)
- Hypochlorhydria (Dundas *et al.* 2001) or previous gastrectomy (Carter *et al.* 1987)
- Low serum albumin three days or less after symptom onset (Dundas *et al.* 2001)
- Antibiotic treatment (Carter *et al.* 1987; Wong *et al.* 2000; Dundas *et al.* 2001)
- Treatment with antimotility agents (Bell *et al.* 1997)
- Gb3 receptor distribution (Lingwood 1996)

The immunological response to VTEC O157 infection has been studied in various populations. Key papers are summarised in Table 4.4.

**Table 4.4 Immunological evidence of immunity to VTEC O157: summary of data from key papers**

Year of publication & author	Type of Study	Methods used	Results	Comments
Chart & Rowe (1992)	Case series of Haemolytic Uraemic Syndrome (HUS) patients.	Detection of IgA-class and IgM-class by ELISA and immunoblotting.	48/125 samples contained IGM-class antibodies to <i>E. coli</i> O157 lipopolysaccharide (LPS) and 42 samples contained IgA-class antibodies. Thirteen samples contained IgM-class but not IgA-class antibodies and seven contained IgA-class but not IgM-class antibodies.	
Siddons & Chapman (1993)	Case series of clinically typical haemorrhagic colitis (HC) cases caused by VT+ <i>E. coli</i> O157 compared with three control groups.	Detection of serum and faecal antibodies in HC caused by VT+ <i>E. coli</i> O157 using EIA for antibodies against <i>E. coli</i> O157.	Marked rise in faecal IgA coincident with cessation of excretion of <i>E. coli</i> O157. Statistically significant differences for <i>E. coli</i> O157 agglutinating antibodies and IgG antibodies against whole cells. No significant difference between cases and controls in detection of serum VT-neutralising antibodies.	
Evans <i>et al.</i> (2000)	Cohort study – PHLS Farmworkers cohort.	1,667 sera from a well-defined cohort examined for antibodies to <i>E. coli</i> O157 LPS by an ELISA. Sera positive for antibodies to <i>E. coli</i> O157 LPS also screened for antibodies to VT1 & VT2.	29/1667 sera (from 22 individuals) were shown to contain antibodies to LPS of <i>E. coli</i> O157, producing ELISA values of >0.4 A <sub>405</sub> . Eleven of 22 individuals had antibodies at enrolment. Seven produced IgA-class antibodies (including one who also produced IgA- and IgM-class antibodies and one who also produced IgA-class antibodies) and four produced IgM-class antibodies. A total of 13 sera from 12 people also contained antibodies to VT. Proportion of O157 antibody-positive individuals was 2.7% at enrolment.	All 22 positive volunteers lived on farms and 20 reported occupational exposure to cattle. Note: those without antibodies also had high levels of exposure to livestock.

Year of publication & author	Type of Study	Methods used	Results	Comments
Currie <i>et al.</i> (2001)	Case series – patients with acute <i>E. coli</i> O157 infection, convalescent <i>E. coli</i> O157 patients, and patients undergoing routine investigation for GI conditions but subsequently shown to be immunologically normal.	Detection of antibodies to <i>E. coli</i> O157 LPS and R3 oligosaccharide in whole gut lavage fluid (WGLF) (IgA-class antibodies), serum (IgG- and IgM-class antibodies) and saliva (IgA-class antibodies) using ELISA.	Patients with acute <i>E. coli</i> O157 infection showed elevated serum levels of IgM-class antibodies to LPS and R3, with IgG-class antibody levels raised only to R3. In serum from convalescent patients IgG-class antibodies were significantly raised above the control groups 6 to 16 weeks after infection. Specific IgA levels were higher in the convalescent group.	Significant IgA-class and IgG-class antibody responses to the R3 core suggest immunological memory.
O'Brien <i>et al.</i> (2001)	Case-control study.	Prospective, unmatched case-control study of 369 patients and 511 controls.	Contact with the farming environment, which included recreational or occupational visits, was strongly associated with sporadic VTEC O157 infection. However, farmers who regularly worked with livestock were not found to be at increased risk.	The fact that farmers who regularly worked with livestock were not found to be at increased risk of developing VTEC O157 infection, yet people who went to farms on work-related or recreational visits were at increased risk, suggests that farmers might have developed immunity to VTEC O157(See also Evans <i>et al.</i> 2000; Silvestro <i>et al.</i> 2004).
Ludwig <i>et al.</i> (2002a)	Case series – 131 children with serologically defined <i>E. coli</i> O157-associated HUS.	Detection of IgA-class, IgG-class and IgM-class antibodies to O157 LPS over time using EIA.	Anti-O157 LPS antibodies decreased below the cut-off levels >50% of children at 11 (IgM), 5 (IgA) and 11 (IgG) weeks after onset of diarrhoea and 10, 4 and 10 weeks, respectively after the onset of HUS.	These children failed to develop a long-lasting humoral immune response to the O157 antigen.

Year of publication & author	Type of Study	Methods used	Results	Comments
Olsen <i>et al.</i> (2002)	Outbreak investigation.	Attack rates amongst town residents and visitors and cross-sectional serological antibody profile examination of a population during an outbreak.	Amongst people exposed to contaminated water (small, unprotected, unchlorinated system) the attack rate was significantly lower in town residents (23%) than in visitors (50%) and decreased with increasing age. People suffering from illness showed markedly increased IgG-class antibodies but not IgM (note: blood samples were obtained two to four weeks after exposure).	

Ludwig and colleagues (2002b) have shown that children with *E. coli* O157 HUS demonstrate a brisk, easily detectable immune response as reflected by the presence of specific antibodies in their saliva, offering a reliable, non-invasive method for the diagnosis of *E. coli* O157 infection in patients with HUS.

## 4.4 Clinical management of VTEC infections

It is generally agreed that the management of VTEC infection and HUS is supportive. Tarr *et al.* (2005) summarised the technical aspects of the clinical management of HUS patients in their recently published review. The most important features when managing VTEC infections are early recognition and the use of intravenous fluids before HUS develops (Ake *et al.* 2005). Early volume expansion, before HUS develops, is associated with better renal outcomes. In practice this means that children in danger of developing HUS need to be admitted to hospital, where their renal function and cardiovascular status can be monitored closely. Antibiotics, anti-motility agents, narcotic drugs, and non-steroidal anti-inflammatory drugs should not be given to acutely infected patients (Tarr *et al.*, 2005). In one study, ciprofloxacin was shown to enhance VT production (VT2c) by a wild-type *E. coli* O157 strain, grown in the presence of this antibiotic (Strauch *et al.* 2004). Ciprofloxacin has also been shown to enhance VT production in strains of VTEC O157: H7 and O111: H<sup>-</sup>, with the greatest increase in a strain of VTEC O157: H7 producing VT2 and VT2c (Baylis 2004). The ability of ciprofloxacin to enhance VT production suggests that this antibiotic should not be used for therapy.

### 4.4.1 Role of antimicrobial agents

Recent information from Denmark states that long term (more than one month), asymptomatic carriers are treated with ciprofloxacin. The treatment is almost exclusively undertaken on patients infected with *eae*-negative VTEC or *eae*-positive VT1 producing strains. The safety of such treatment needs to be tested or verified by other groups. In addition, the spectrum of VTEC virulence types that may be treated safely needs to be investigated.

In the USA, patients infected by VTEC O157 in some hospitals are normally already started on antimicrobial therapy and normally receiving daily plasmapheresis when the infectious disease team are consulted to see the patient. Consensus of opinion in one large teaching hospital was that the evidence did not indicate that treatment was either harmful or beneficial. It is therefore important that definitive guidance supported by scientific evidence is provided to medical staff treating patients infected with VTEC (Dr. K.J. Dodgson, personal communication).

The balance of opinion is against the use of antimicrobial agents to treat VTEC infection since evidence points to an increased risk of HUS following antibiotic treatment (Table 4.5).

**Table 4.5 Two recent studies demonstrating an increased risk of HUS following antibiotic treatment**

Year	Type of study	Strength of Association	Comments	Reference
1996	Retrospective, hospital-based study following a large outbreak	Adjusted Odds Ratio (OR) = 4.7; 95% Confidence Interval (CI) = 1.4-16.5	Limitations include accuracy and completeness of medical record keeping at the time of the outbreak.	Dundas <i>et al.</i> , (2001)
?	Prospective cohort study in children < 10 years of age	Relative risk (RR) = 17.3; 95% CI = 2.2 to 137	Limitations include selection bias and potential confounding	Wong <i>et al.</i> , (2000)

Ikeda *et al.* (1999) suggested that treatment with fosfomycin in the first two days of VTEC O157 illness might prevent the development of HUS.

Safdar *et al.* (2002) published a meta-analysis of published studies and concluded that there was no evidence of an increased risk of HUS following antibiotic treatment. However, there were several criticisms of the methods they used to reach this conclusion and the weight of opinion is still that antibiotics should not be used.

Intriguingly, there are two recently published examples where antibiotics have been used to eliminate carriage of VTEC O157. Jensen *et al.* (2005) report the outcome of an empirical study, in which nine asymptomatic people, whose activity had been severely restricted for prolonged periods due to official quarantine, were treated with ciprofloxacin or ampicillin. In all nine instances treatment was successful and without complications. Eight of the nine VTEC strains causing infection did not carry the *eae* gene. Spacek *et al.* (2004) have also treated successfully two patients in whom VTEC O157 was thought to be the cause of chronic diarrhoea.

#### 4.4.2 Vaccines

Ahmed *et al.* (2006) published the results of a phase 2 clinical trial of an O-specific polysaccharide conjugate vaccine. The vaccine consists of *E. coli* O157: H7 O-specific polysaccharide conjugated to recombinant exotoxin A of *Pseudomonas aeruginosa* (O157-rEPA) and they have trialled its use in children aged 2 to 5 years. Children were randomised to receive one or two doses of the candidate vaccine and 55 completed the trial. The O157-rEPA vaccine was safe and immunogenic in young children. All children had low

serum IgG LPS antibody titres prior to vaccination and most of the children showed a >10-fold increase in serum IgG LPS antibody titres 6 weeks after the first dose. Furthermore at 26 weeks after the first dose, their serum IgG LPS antibody titres remained significantly higher than the pre-vaccination titres. A second dose of the vaccine did not induce a booster response. A phase 3 trial is planned.

Wen *et al.* (2006) have developed a plant-based oral vaccine by genetically inactivating the VT2 active A subunit gene and optimizing both subunit genes for expression in plants. Oral immunization fully protected mice from subsequent challenge with *E. coli* O91: H21 strain B2F1, showing that the plant-based oral vaccine protected against lethal systemic intoxication. However, further work on vaccine development is still needed before clinical trials could take place in man.

Marcato *et al.* (2005) have used a recombinant VT2 B subunit vaccine containing <1 endotoxin unit per ml in mice. As well as mounting a specific immune response, all the vaccinated mice were protected from a lethal dose of VT2 holotoxin. The researchers argue that further evaluation of a VT2 B-subunit-based human EHEC vaccine is needed.

Using a different approach, Mayr *et al.* (2005) developed an oral EHEC vaccine by protein E-mediated cell lysis to produce EHEC ghosts combined with staphylococcal nuclease A to degrade DNA, thereby eliminating any hazard of horizontal gene transfer of resistance genes or pathogenic islands to resident gut flora. A high proportion of vaccinated mice showed protection against lethal challenge with a heterologous EHEC strain after single-dose oral vaccination and a booster response was clearly demonstrated.

#### **4.4.3 Alternative therapies**

A number of therapeutic avenues has been explored, as yet without success, including: -

- SYNSORB: oral agent designed to bind Shiga toxin in the gut. In a multi-centre, randomised, double-blind, placebo-controlled trial, oral therapy with this agent failed to diminish the severity of disease in children with diarrhoea-associated HUS (Trachtman *et al.* 2003).
- Gb3 polymer: oral agent designed to bind VT in the gut. Promising results in mice but not yet ready for trials in humans (Karmali 2004; Watanabe *et al.* 2004).
- Medicinal plants: a variety of medicinal plants has been shown to have antibacterial activity and might, in the future, provide alternative

bioactive medicines for the treatment of VTEC O157 infection (Voravuthikunchai *et al.* 2004).

- Probiotic bifidobacteria: promising results reported in mice that were protected from developing HUS (Asahara *et al.* 2004). Clinical trials in humans have not been published.
- Antibody therapy: a VT2-specific human monoclonal antibody with a wide spectrum of activity against VT2 and VT2 variants has been produced and is anticipated to commence clinical trials shortly (Tzipori *et al.* 2004).
- Plasmapheresis: Dundas and Todd (2000) reported that plasmapheresis was a useful therapeutic tool in treating adult HUS cases resulting from the Central Scotland outbreak of *E. coli* O157. However, the number of patients treated was very small. Tarr *et al.* (2005) have expressed the view that there is no theoretical justification for this type of intervention in VTEC-related HUS, where there is no evidence of ADAMTS13 deficiencies (see Section 4.2 and 4.3) or any other removable or replaceable factor.
- Vaccines: there are no candidate vaccines in humans yet.
- Altering the susceptibility of intestinal flora to attack by *vtx* encoding phage could be used to reduce the risk of severe disease developing but this approach and the role of intestinal flora requires further investigation.

#### **4.5 Gaps in knowledge**

- After several years of routine serodiagnosis we still have only a poor understanding of the kinetics of antibody production by patients infected with *E. coli* O157.
- Diagnostic criteria for VTEC infection need to be standardised internationally and certainly at the EU level.
- Studies of the population and environmental determinants of the occurrence of VTEC infection are required.
- The extent of, and public health significance of, asymptomatic VTEC carriage is unknown. Cases and their close contacts need to be followed up systematically in order to understand these better. One option would be to undertake a detailed follow-up of every case reported over a twelve month period.

- Further data on the potential use of antimicrobials to eliminate carriage, especially in those who pose a special risk for spread to others, are needed. The two case studies reported so far are very small, but the circumstances under which a trial might be undertaken need careful consideration.
- The consequences of VTEC O157 have been studied extensively in the child population – not surprisingly since they are at greatest risk. However, this means that data on clinical course and outcome are lacking in adults. A national disease register of VTEC cases, such as exists already in Scotland, should be established in England and Wales.
- There needs to be a better understanding of how GPs and Paediatricians approach the management of bloody diarrhoea, especially in children.
- In the UK, there needs to be an assessment of the role of non-O157 VTEC in diarrhoeal disease. It is not usually found; however, it is not often sought.
- Further data are needed on the potential role of probiotics in disease management.
- Recent information from Denmark states that long term (more than one month) asymptomatic carriers are treated with ciprofloxacin. The safety of such treatment needs to be tested or verified by other groups.
- The spectrum of VTEC virulence types and the safety of antibiotic treatment needs to be investigated.
- Despite the universal agreement that antimicrobial therapy during infection is contraindicated, more definitive guidance and study is required. This is hampered by the sporadic nature of infection and outbreaks. The guidelines from the British Society for Haematology (Br. J. Haematology, 2003) address the use of anti-motility agents and plasma exchange but only briefly mention risk data regarding antimicrobial therapy in children (Wong *et al.*, 2000). The lack of definitive guidelines needs to be addressed.

- Despite the UK recommendation that all stools be inoculated on CT-SMAC to screen for O157 it would be useful to see how many laboratories still perform this recommended procedure and due to low/no prevalence and time/money constraints.

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