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REVIEW ARTICLE

Escherichia coli serogroup O26 – a new look at an old adversary

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Summary

Escherichia coli serogroup O26 played an important part in the early work on Verocytotoxin and is an established diarrhoeal pathogen. Recently, Verocytotoxigenic *E. coli* (VTEC) O26 has been increasingly associated with diarrhoeal disease and frequently linked to outbreaks and cases of haemolytic uraemic syndrome (HUS). This review investigates the pathogenicity, geographical distribution, changing epidemiology, routes of transmission and improved detection of VTEC O26. Laboratory data on VTEC O26 isolates and clinical data on HUS suggest a true difference in the incidence of VTEC O26 in different geographic locations. However, few diagnostic laboratories use molecular methods to detect VTEC and so it is difficult to assess the role of VTEC O26 in causing diarrhoeal disease. VTEC O26 is frequently found in the cattle population but rarely in food. However, the small number of outbreaks analysed to date are thought to be food-borne rather than associated with direct or indirect contact with livestock or their faeces. The increase in awareness of VTEC O26 in the clinical and veterinary setting has coincided with the development of novel techniques that have improved our ability to detect and characterize this pathogen.

Introduction

Although part of a large group of *Escherichia coli* serotypes and pathotypes associated with diarrhoea (Nataro and Kaper 1998; Bettelheim 2000), Verocytotoxin-producing *E. coli* (VTEC) O157:H7, also referred to as Shiga toxin-producing *E. coli* (STEC) and enterohaemorrhagic *E. coli* (EHEC), has monopolized the headlines since the early 1980s (Riley *et al.* 1983; Morgan *et al.* 1993; Upton and Coia 1994; Pritchard *et al.* 2000; Dobson 2006). This notoriety is justified as this serotype has caused frequent outbreaks [44 food-borne outbreaks between 1992 and 2002 in England and Wales (Gillespie *et al.* 2005)], some involving large numbers of people (Cowden *et al.* 2001), and causing severe disease (Lynn *et al.* 2005). However, the emergence of VTEC O26 predates that of all other VTEC and this serogroup played an essential part in the early work on Verocytotoxin (vtx) (Smith and Linggood 1971; Scotland *et al.* 1983).

VTEC O26 has featured in many studies of diarrhoeal disease over the last 25 years (Table 1) (Scotland *et al.* 1980, 1993; Willshaw *et al.* 1992; Evans *et al.* 2002), and in countries where tests for VTEC include serogroups other than O157, the incidence of VTEC O26 is similar to that of O157 (Caprioli *et al.* 1997; Schmidt *et al.* 1999). In many countries, including the United Kingdom, there are no standard methods for the detection of non-O157 VTEC in routine diagnostic laboratories, with VTEC protocols focussing on the use of cefixine tellurite sorbitol MacConkey (CT-SMAC) agar (March and Ratnam 1986; Chapman *et al.* 1991). Unlike VTEC O157, VTEC O26 is not selected by this medium as it generally ferments sorbitol, and this may contribute to the incidence of VTEC O26 being much lower than O157

Table 1 Details of data from studies on *Escherichia coli* O26 between 1980 and 2003

Reference	Findings
Scotland *et al.* (1980)	Verocytotoxin (vtx) was produced by 23 of 54 strains of *E. coli* O26 held in the culture collection at the Laboratory of Enteric Pathogens (LEP)
Scotland *et al.* (1990)	Twenty-seven of 37 strains of *E. coli* O26, held in the culture collection at the LEP had *vtx* genes, including four strains from haemolytic uraemic syndrome (HUS) patients
Scotland *et al.* (1990)	Sixteen of 25 strains of *E. coli* O26 from children with diarrhoea, and seven of eight strains from calves belonged to the Verocytotoxigenic *E. coli* (VTEC) pathotype. Of these 27 strains, 26 had *vtx*1 genes, one had genes encoding *vtx*2 (from a patient with HUS)
Scotland *et al.* (1990)	Twenty-nine of the 37 LEP *E. coli* O26 strains had the enterohaemolysin (*ehx*A) genes, 24 also produced vtx whereas five did not. Sixteen strains were analysed for the presence of plasmids, of which 15 carried between one and four plasmids. The size of the plasmid hybridizing with the CVD419 probe varied, although the size of the plasmid did not correlate with the source of the strains or with any other property
Willshaw *et al.* (2001) Jenkins *et al.* (2003a)	Nine of sixty-two non-O157 VTEC isolates from patients with HUS and diarrhoea sent to the LEP between 1983 and 2000 from patients resident in the United Kingdom, were VTEC O26:H11 or O26:H-. All of these isolates had the *vtx*1 and *eae* genotypes
Evans *et al.* (2002)	During a study of community-acquired intestinal infectious disease (IID) in the United Kingdom, 27 strains of VTEC were isolated, including one strain of VTEC O26:H11
Scottish Centre for Infection and Environmental Health (SCIEH) (2003)	Between June and August 2003, the Scottish *E. coli* O157 Reference Laboratory (SERL) reported the isolation of five strains of non-O157 VTEC. Two strains were from siblings from Ayrshire and Arran, one from Forth Valley, one from Lothian and one from Tayside. Four isolates were identified as O26, *vtx*1 and one as O113. Pulsed field gel electrophoresis (PFGE) identified different patterns from each NHS board area
McMaster *et al.* (2001)	Microbiology showed lactose-fermenting colonies from stool samples that agglutinated with *E. coli* O26 sera, and these were later confirmed as *E. coli* serotype O26:H11, *vtx*1 only. Screening of staff (n = 5) and other children (n = 29) identified four other infected people. Analysis of seven isolates by PFGE showed four identical patterns that differed by only one band from the three other isolates that were identical to each other. All isolates had intimin (*eae*) and enterohaemolysin (*ehx*A) genes
Hiruta *et al.* (2001)	Sixteen O26 strains were isolated from 32 children with diarrhoea; all had *vtx*1, *eae* and *ehx*A genotypes. VTEC O26 was also isolated from two food samples consisting of mixed vegetables with bean sprouts and sliced watermelon. Interestingly, VTEC and non-VTEC, *eae*-negative *E. coli* O26 strains were also isolated from six of the children. The *vtx*-positive, *eae*-negative isolates had the outbreak in the PFGE pattern whereas the non-VTEC, *eae*-negative strains had a different pattern
Weber *et al.* (2002)	All 18 isolates in this multistate outbreak in Germany harboured *vtx*1, *eae* and *ehx*A. Beef was suggested as a possible source although there was no bacteriological evidence for this

(Willshaw *et al.* 2001). Recently, VTEC O26 has been increasingly associated with diarrhoeal disease (SCIEH 2003; Carroll *et al.* 2005), and frequently linked to outbreaks (Hiruta *et al.* 2001; McMaster *et al.* 2001; Weber *et al.* 2002) and cases of haemolytic uraemic syndrome (HUS) (Liptakova *et al.* 2005; Sayers *et al.* 2006).

The ability of strains of VTEC to colonize humans and animal intestinal mucosa, and to cause disease, is associated with a number of virulence factors, including the expression of Verocytotoxins (vtx) (reviewed in Tarr *et al.* 2005) and, in certain serogroups including O26 and O157, the ability to induce attaching/effacing (A/E) lesions (reviewed in Frankel *et al.* 1998; Chen and Frankel 2005). Recent studies have highlighted interesting differences, and similarities, between the molecular mechanisms of pathogenesis exhibited by VTEC O26 and O157 (Campellone and Leong 2003; Caron *et al.* 2006).

The source of the outbreaks or sporadic cases of VTEC O26 described in the literature is rarely found (Zhang *et al.* 2000). However, VTEC O26 has been isolated from the faeces of cattle, calves, pigs, lambs and goats (Leomil *et al.* 2005) and transmission of VTEC O26 directly or indirectly from animals and their faeces is a possible route of infection. Recent farm studies on the epidemiology and shedding patterns of VTEC O26 have produced data on the risk factors associated with VTEC human infection (Pearce *et al.* 2004; Shaw *et al.* 2004; van Diemen *et al.* 2005; Lui *et al.* 2005). Food-borne transmission is frequently associated with infection with VTEC O157, and serogroup O26 has been detected in meat and dairy products (Steele *et al.* 1997; Hussein and Sukuma 2005; Murphy *et al.* 2005).

There are a large number of studies reporting the presence of VTEC O26 in both healthy and diarrhoeic calves, whereas others have found no such association (Mohammad *et al.* 1985; Sherwood *et al.* 1985; Orden *et al.* 1998; Cobbold and Desmarchelier 2000; Pearce *et al.* 2004). The characteristics of VTEC O26 that may facilitate colonization of the bovine gut are currently under investigation (Roe *et al.* 2001; Shaw *et al.* 2004; van Diemen *et al.* 2005).

The increase in awareness of VTEC O26 in both the clinical and veterinary setting has coincided with the development of selective media and improvements in the specificity and sensitivity of the methods for detecting and isolating VTEC O26. This review aims to evaluate the historical, epidemiological and novel molecular data on VTEC serogroup O26, and to discuss issues such as: (i) molecular mechanisms of pathogenesis; (ii) geographical distribution; (iii) changing epidemiology; (iv) routes of transmission; (v) pathogenicity in calves; (vi) improved detection and (vii) phylogenetic relationships of VTEC O26 with other *E. coli* pathogens associated with diarrhoea.

Historical background

Theodor Escherich, an Austrian paediatrician, described *E. coli* in 1885, calling it *Bacterium coli commune* to reflect its universal occurrence in the faeces of healthy individuals (Escherich 1885). However, it was not until 1939 that the relationship between *E. coli* and infantile diarrhoea was fully elucidated (Bray 1945). Kauffmann's *E. coli* serotyping scheme, based on the somatic lipopolysaccharide or 'O' antigen and the flagella or 'H' antigen, helped clarify the serogroups more likely to be associated with the disease (Kauffmann 1947). In North America at that time, investigations of hospital outbreaks of infantile diarrhoea in New York showed that these *E. coli*, now referred to as enteropathogenic *E. coli* (or EPEC), belonged to a limited set of O serogroups, notably O26, O55 and O111 (Ewing *et al.* 1963).

The mechanisms involved in EPEC pathogenicity are now fully understood (Frankel *et al.* 1998) but during the 1960s and 1970s, research focussed on the possibility that EPEC, like the enterotoxigenic *E. coli*, produced an enterotoxin. In 1971, a seminal paper was published that examined human strains of EPEC, using the rabbit-ligated intestine technique, to determine whether they produced enterotoxin (Smith and Linggood 1971). Only one strain, H19 serotype O26:H11, produced a toxin, and this strain is now known to be the first strain of VTEC to be described in the literature.

Verocytotoxin was first described by Konawalchuk *et al.* (1977) as a cytotoxin that 'induced a distinctive cytotoxic effect on Vero that was different and easily distinguished from that produced by heat-labile enterotoxin'. Verocytotoxin was cytotoxic for Vero cells but not for Y-1 or CHO cells, heat-labile but antigenically different from heat-labile enterotoxin, and had a molecular weight of 10 000–30 000. Among the strains analysed in this study were two isolates of *E. coli* O26, from human infants with diarrhoea, one of which was strain H19. Scotland *et al.* (1980) subsequently found that vtx was produced by 23 of 54 strains of *E. coli* O26 held in the culture collection at the Laboratory of Enteric Pathogens (LEP), Health Protection Agency (HPA) (then the Central Public Health Laboratory), London. The fact that vtx was mediated by a bacteriophage and could be transferred between strains was first demonstrated in a strain of VTEC O26 (Scotland *et al.* 1983).

The clinical relevance of vtx was demonstrated in 1983, when Karmali *et al.* (1983) showed that VTEC was the causative agent of HUS. VTEC was isolated from 8 of 15 patients and two HUS contacts, and vtx-neutralizing antibodies were detected in four additional patients. The strains of VTEC in this study belonged to at least four different serogroups, including O26, O111, O113 and O157 (Karmali *et al.* 1983). In the early 1980s, two large outbreaks of bloody diarrhoea in the United States were found to be caused by VTEC O157:H7 and methods for detecting VTEC focussed on this serotype, particularly in the United States and United Kingdom (Riley *et al.* 1983; Wells *et al.* 1983).

Pathogenic mechanisms

EPEC have the ability to form A/E lesions on the intestinal epithelium. A/E lesions are characterized by intimate bacterial attachment to the host cell membrane and the destruction of microvilli at the site of bacterial adherence, caused by the accumulation of signal proteins leading to the rearrangement of cytoskeletal proteins, in particular filamentous actin, resulting in pedestal formation at the apical cell membrane (Frankel *et al.* 1998; Chen and Frankel 2005). The genes necessary for the formation of A/E lesions are located on a 35-kb chromosomal region called the locus of enterocyte effacement (LEE). One of the genes (*eae*, for *E. coli* attaching and effacing) on the LEE region encodes an outer membrane adhesin protein termed intimin (Jerse *et al.* 1990). Classical EPEC, including strains belonging to serogroups O55, O111 and O127, are characterized by the presence of the EPEC adherence factor (EAF) plasmid (Cravioto *et al.* 1979). EPEC lacking the EAF plasmid are sometimes referred to as nonclassical, atypical or A/E *E. coli* (AEEC) (Trabulsi *et al.* 2002). EPEC O26 does not harbour the EAF plasmid, although it is one of the most important EPEC

serogroups and was among the first to be considered a cause of infantile diarrhoea (Neter *et al.* 1955). It has not, however, been associated with outbreaks of infantile diarrhoea (Neter *et al.* 1955; Smith *et al.* 1996).

Formation of the A/E lesion is essential for EPEC pathogenicity and these lesions have also been associated with other bacterial pathogens, including certain VTEC, such as those belonging to serogroups O157 and O26 (Tzipori *et al.* 1989). Studies show that there are no gross differences between lesions caused by VTEC O26 and those caused by EPEC O26 (Tzipori *et al.* 1989; Aktan *et al.* 2004). However, there is evidence that there are some differences at the molecular level (Campellone and Leong 2003; Caron *et al.* 2006; Ogura *et al.* 2007).

Central to the formation of A/E lesions is the ability of the bacteria to polymerize actin in the host cells in the human gut mucosa to produce elongated, actin-rich pedestal-like structures under the attached bacteria (Knutton *et al.* 1987). The genes required for formation of A/E lesions by VTEC O157 are also carried on the LEE pathogenicity island but those on the prophage CP-933U are also necessary (Garmendia *et al.* 2004). CP-933U carries a gene encoding the Tir-cytoskeleton coupling protein (TccP), that is also labelled $EspF_u$, which is required for actin polymerization in VTEC O157. Actin polymerization by EPEC (serogroup O127:H6) requires the phosphorylation of translocated intimin receptor (Tir), which forms a binding site for an adaptor protein, Nck, whereas Tir in VTEC O157 is not phosphorylated and uses TccP rather than Nck (Campellone and Leong 2003; Caron *et al.* 2006). The LEE of VTEC O26 contains all the genes needed for A/E lesions, and a homologue of $TccP_{O157}$, TccP2, has also been found in VTEC O26 (Garmendia *et al.* 2005). Recent studies suggest that actin assembly by VTEC O26 can be mediated by either the phosphorylation of Tir, similar to EPEC O127:H6, or via the TccP pathway as seen in VTEC O157 (Ogura *et al.* 2007). This versatility may enable VTEC O26 to interact with a wide range of hosts and cell types.

Like VTEC O157, serogroup VTEC O26 strains can elaborate vtx1, vtx2 or both (O'Loughlin and Robins-Browne 2001; Karmali 2004; Tarr *et al.* 2005). The definitions of vtx and the organisms that produce them are reviewed in Tarr *et al.* (2005). vtx1 and 2 are a family of toxins, including subtypes vtx1, 1c, 2, 2c, 2d, 2e and 2f. Subtypes 1, 2 and 2c have been detected in VTEC O26 (Jenkins *et al.* 2003a). The toxins share a polypeptide subunit structure consisting of an enzymatically active A subunit (32 kDa) that is linked to a pentamer B subunit (7·5 kDa) (O'Loughlin and Robins-Browne 2001). Eukartyotic cells contain a glycolipid called globotriosylceramide (Gb3) that acts as a receptor for vtx. The toxin is then taken into the cell by endocytosis and transported to the endoplasmic reticulum where it inhibits protein synthesis by preventing aminoacetyl tRNA from binding to the ribosome causing cellular damage (O'Loughlin and Robins-Browne 2001; Karmali 2004). Destruction of the endothelial cells in the intestine results in diarrhoea. In cases of HUS, vtx is transported via the blood stream and targets cells in the kidney, brain, pancreas and lungs causing widespread thrombotic microangiopathy (Karmali 2004).

VTEC O26 possess large plasmids that contain genes encoding potential virulence factors including an enterohaemolysin, which acts as a pore-forming cytolysin (Schmidt *et al.* 1995; Brunder *et al.* 1999). Beutin *et al.* (1989) showed that the presence of *vtx* genes in VTEC O26 correlated well with the presence of *ehx*A genes. Other genes present on the large plasmids include *katP*, which encodes a catalase-peroxidase (Brunder *et al.* 1996) and a serine protease (EspP) that cleaves human coagulation factor V (Brunder *et al.* 1997). Recent work has demonstrated that this plasmid also harbours a 10-kb virulence gene designated *toxB* in VTEC O157 and that DNA sequences related to *toxB* have been detected in VTEC O26 (Tozzoli *et al.* 2005). It has been shown that VTEC O26 contains a high pathogenicity island (HPI) also found in pathogenic *Yersinia* species, but not present in VTEC O157 (Karch *et al.* 1999; Zhang *et al.* 2000).

Incidence and epidemiology

The incidence of VTEC O26-associated disease is probably underestimated because of diagnostic limitations (Carroll *et al.* 2005). Although the incidence of VTEC O26 remains low in relation to VTEC O157 in the United Kingdom, VTEC O26 has emerged as a particularly significant cause of human disease in other countries (Caprioli *et al.* 1997; Schmidt *et al.* 1999; Tozzi *et al.* 2003; Carroll *et al.* 2005) (Fig. 1).

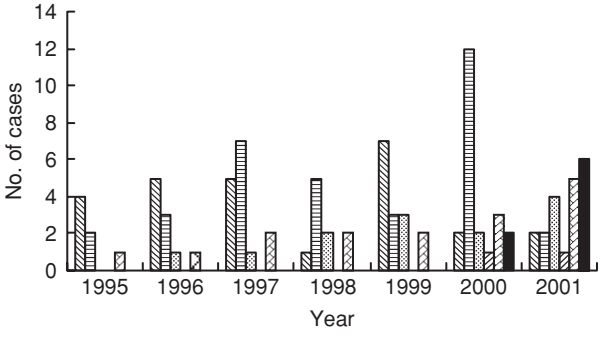

Figure 1 Serogroups of Verocytotoxigenic *Escherichia coli* (VTEC) in Italy: 1995–2001 (Smith *et al.* 2002). ▨, O157; ▤, O26; ▩, O103; ▨, O111; ▨, O145; ■, O?.

VTEC O26 strains can produce *vtx*1, *vtx*2 or both. Zhang *et al.* (2000) showed that *vtx*1 was the exclusive O26 genotype identified until 1994 in Germany but was only rarely detected between 1997 and 1999. They suggested that this change may be because of the emergence of a *vtx*2-harbouring clonal subgroup whose dominance may be related to better adaptation to the host or to changes in food consumption. Analysis of the VTEC O26 strains harbouring *vtx*2 genes either alone or in combination with *vtx*1 from the culture collection at the LEP (HPA, London), showed that there has been an increase in *vtx*2-producing strains between 1994 and 2002 (Fig. 2). These strains originated from Ireland, Scotland, other parts of Europe and from UK patients who had recently travelled abroad. Strains associated with human disease and in particular HUS more commonly carry *vtx*2 or *vtx*1 and 2, rather than *vtx*1 only (Bonnet *et al.* 1998; Friedrich *et al.* 2002; Jenkins *et al.* 2003a) and this has been seen recently in two cases of HUS. In the first case the isolate of VTEC O26 had *vtx*2 only (Liptakova *et al.* 2005) and the strains associated with the second case had both *vtx*1 and 2 genes (Sayers *et al.* 2006). As *vtx*2 is more commonly associated with severe disease, it is possible that this genotype shift has caused an increase in cases of HUS associated with VTEC O26.

In a prospective study on the clinical course and the role of VTEC in HUS in paediatric patients in Germany and Austria, non-O157 VTEC accounted for almost half of the isolates (90 of 207 strains). Thirty-one (15%) strains of VTEC O26 were isolated and this serogroup was the most common non-O157 VTEC detected (Gerber *et al.* 2002). This contrasts with United Kingdom, where 83% of the cases of childhood HUS are caused by VTEC O157:H7 (Lynn *et al.* 2005). Non-O157 VTEC were not sought in this study and it is a potential criticism that non-O157 VTEC strains might have been missed but the fact remains that the vast majority of cases had evidence of infection with *E. coli* O157.

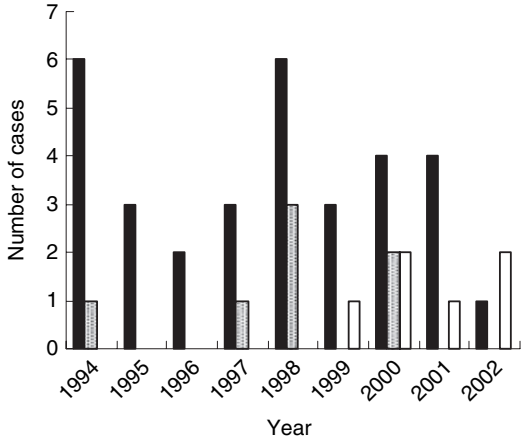

Figure 2 *vtx* genotype of Verocytotoxigenic *Escherichia coli* (VTEC) O26 in the Laboratory of Enteric Pathogens collection between 1994 and 2002. ■, vtx 1; ▨, vtx 2; □, vtx 1+2.

Outbreaks and routes of transmission

Recently, a number of case studies of outbreaks of VTEC O26 have been published. A VTEC O26 outbreak occurred in a nursery in Japan in July 1997 (Hiruta *et al.* 2001) involving 32 children with diarrhoea, and in Germany, in March/April 2000, 18 VTEC O26 isolates were isolated from a multistate outbreak (Weber *et al.* 2002) (Table 1).

McMaster *et al.* (2001) described four cases of diarrhoea in children attending a crèche in rural NW Ireland. Despite having symptoms of nonbloody diarrhoea, vomiting, pyrexia and abdominal cramps, the children had continued to attend the crèche (Table 1). Facilities for food preparation and storage were found to be unacceptable but a specific vehicle of infection was not found.

Evidence from the outbreaks described before suggests that food was the most likely route of transmission and this was confirmed using pulsed field gel electrophoresis (PFGE) to compare strains in the study by Hiruta *et al.* (2001). PFGE was also used to link two cases of HUS caused by VTEC O26 in Austria with the consumption of unpasteurized cow's milk (Allerberger *et al.* 2003).

Although the use of selective media, serogroup-specific immunomagnetic separation techniques (IMS) and polymerase chain reaction (PCR) have improved the detection of VTEC O26 in food (Drysdale *et al.* 2004; Murinda *et al.* 2004; O'Hanlon *et al.* 2004), this serotype is rarely detected (Hussein and Sukuma 2005). Murphy *et al.* (2005) detected VTEC O26 in 0·25% of minced beef samples in Ireland. In Australia, studies showed that VTEC was identified in 16% of ground beef and 40% of lamb using PCR but VTEC serogroups O26, O111 and O157 were not isolated (Barlow *et al.* 2006). In studies of VTEC in cattle and beef products in Argentina, VTEC O26 was detected in grazing cattle but not in meat (Blanco *et al.* 2004) and results of a Serbian study concluded that VTEC 'was prevalent in animals, and to a lesser extent in food of animal origin' (Cobeljic *et al.* 2005).

Strains of VTEC O26 have been isolated from healthy cattle and calves, calves with diarrhoea, pigs, lambs, goats and chicken (Leomil *et al.* 2005). In 1990, Scotland *et al.* (1990) concluded that '[t]here has been recent discussion on the zoonotic nature of the reservoir of strains causing haemorrhagic colitis and whether the strains can cause human and animal disease. *E. coli* isolated from animals and humans have similar characteristics, supporting the probability that animals are a source of this serotype'.

VTEC O26 in cattle

Escherichia coli O26 has been detected in calves and cattle since the early 1980s (Sherwood *et al.* 1985; Pearson *et al.* 1999; Geue *et al.* 2002). Orden *et al.* (1998) found that the most common VTEC serogroup in calves was VTEC O26 (30%). Cobbold and Desmarchelier (2000) looked for VTEC on three farms in Australia and found that 7·8% of the isolates were VTEC O26:H11.

Models of the epidemiology of VTEC in calves show that some VTEC serogroups are transient (duration of infection of <1 day) while others have a longer duration of infection (3–5 days), thus facilitating their ability to persist in the herd (Lui *et al.* 2005). In the study by Shaw *et al.* (2004), the most frequently isolated VTEC serogroup in calves was VTEC O26 and the authors suggest that characteristics of this serogroup, such as possession of the β-intimin gene, may influence its ability to colonize and persist in young cattle. Extensive adherence and A/E lesion formation occur with VTEC O26 in the bovine large intestine whereas VTEC O157 adheres sparsely to the large intestinal mucosa in calves and exhibits tropism for lymphoid follicle-dense mucosa in the terminal rectum of older animals, suggesting that VTEC O157 and O26 may colonize the bovine intestine by distinct mechanisms (Naylor *et al.* 2003; van Diemen *et al.* 2005; Ogura *et al.* 2007).

The screening of random mutants in calves has shown that VTEC O26:H- uses some of the same factors as VTEC O157 to colonize calves, such as the LEE-encoded type III proteins, as well as some serotype-specific factors, including cytotoxins, type III secreted proteins unlinked to the LEE and genes involved in metabolism and transport (van Diemen *et al*. 2005). Other studies have demonstrated that type 1 fimbriae adhere well to the rumen epithelium (Galfi *et al.* 1998) and that, although the majority of VTEC O157 do not express type 1 fimbriae, VTEC O26 can express this adhesin (Enami *et al.* 1999). Certain studies have suggested that the expression of type 1 fimbriae by *E. coli* O26 could enhance colonization in the bovine host and may explain why VTEC O26 persist in cattle (Roe *et al.* 2001), while others show that type 1 fimbriation is selected against during infection of cattle (van Diemen *et al*. 2005).

VTEC O26 is commonly found in faecal samples of healthy calves and cattle, and calves with diarrhoea (Mohammad *et al.* 1985; Sherwood *et al.* 1985; Cobbold and Desmarchelier 2000; Pearce *et al.* 2004). A significant association between VTEC and diarrhoea in calves has been described but other studies have not been able to detect an association (Mohammad *et al.* 1985; Orden *et al.* 1998; Cobbold and Desmarchelier 2000; Pearce *et al.* 2004). In the study by Pearce *et al.* (2004), a link between shedding VTEC O26 and scouring in calves was not demonstrated. Given the level of VTEC O26 shedding described in this study, it was suggested that dams were transferring protective antibodies to their calves via colostrum and thereby preventing diarrhoeal disease (Pearce *et al.* 2004).

Detection of *Escherichia coli* O26

Unlike the majority of faecal *E. coli*, most strains of VTEC O157 are unable to ferment sorbitol and this characteristic has been exploited to differentiate VTEC O157 strains from other faecal *E. coli*. The use of selective media has, without doubt, improved the detection of VTEC O157. The carbohydrate-fermenting ability of VTEC O26 has been investigated and a rhamnose MacConkey medium containing cefixime and tellurite (CT-RMAC) has been developed (Hiramatsu *et al.* 2002; Murinda *et al.* 2004). VTEC O26 strains generate colourless colonies (rhamnose-nonfermenters) in contrast to other *E. coli* strains, which appear as red-coloured colonies. Like O157, O26 is resistant to tellurite, which suppresses the growth of most gram-negative organisms and cefixime suppresses *Proteus* species. Catarame *et al.* (2003) found that CT-RMAC was the optimum agar for recovery of *E. coli* O26. They also found that optimum enrichment conditions were seen in tryptone soya broth supplemented with cefixime, vancomycin and potassium tellurite incubated at 41°C, whereas Hara-Kudo *et al.* (2000) found that modified *E. coli* broth with novobiocin was the most effective.

Enterohaemolysin and chromogenic agars have been used to recover VTEC O26 from food and faecal samples (Beutin *et al.* 1996; Hara-Kudo *et al.* 2000; Novicki *et al.* 2000). VTEC O157 appears as black-coloured colonies, whereas other *E. coli*, including VTEC O26, appear as red, blue or purple colonies. These agars are useful as a screening method but must be used in conjunction with more specific tests such as enzyme-linked immunosorbent assay (ELISA) that detect the presence of *vtx* or serogroup-specific immunomagnetic separation techniques (IMS), as the VTEC strains are indistinguishable from the *vtx*-negative strains (Bettelheim 1998).

Serogroup-specific IMS techniques are an effective way of isolating *E. coli* O26 from faeces (Pearce *et al.* 2004) and food (Drysdale *et al.* 2004) and have been shown to be 2·5 times more sensitive than conventional PCR/DNA probe techniques, although IMS is not specific for VTEC (Jenkins *et al.* 2003b). PCR or DNA probe techniques that detect the presence of *vtx* and/or *eae* genes can be used to detect VTEC, including VTEC O26, although they are not specific for VTEC O26 (Louie *et al.* 1998; Willshaw *et al.* 2001). Other PCR assays, targeting genes found in the O26 O-antigen gene cluster such as the

O-unit flippase (*wzx*) and the O-antigen polymerase (*wzy*) (D'Souza *et al.* 2002; DebRoy *et al* 2004) have been used for the detection of O26 in clinical cases (Perelle *et al.* 2004) and food (O'Hanlon *et al.* 2004). ELISA detecting the presence of *vtx*1 and *vtx*2 have been used to detect VTEC (Paton and Paton 2003), and more recently, monoclonal antibodies against O26 surface antigens have been used in an ELISA format for the specific detection of *E. coli* O26 (Neely *et al.* 2004) and compared favourably with IMS (Finlay *et al.* 2006).

Molecular techniques for characterizing *E. coli* O26

Zhang *et al.* (2000) suggested that to identify sources and modes of spreading, VTEC O26 isolates need to be subtyped to provide adequate strain discrimination. Whittam *et al.* (1993) examined 93 VTEC O26 isolates by multilocus enzyme electrophoresis (MLEE) typing. They differentiated 20 electrophoretic types although most isolates belonged to one of two types termed DEC9 and DEC10. Random-amplified polymorphic DNA (RAPD) analysis was used to type 34 strains of *E. coli* O26 including EPEC and VTEC strains of different geographic origin (Peixoto *et al.* 2001). Both EPEC and VTEC strains were found in the same cluster and the authors concluded that 'although the expression of some important phenotypic characteristics depend on specific minor genetic differences, like plasmid and phage acquisition, the overall genomic background plays a significant role in determining the evolution of a pathogenic clonal sub-group' (Peixoto *et al.* 2001).

The relationship between strains in the *E. coli* O26 group compared with other *E. coli* isolates was investigated further using comparative genomic indexing to define the core genes common to all strains but also to identify regions of difference between these strains (Anjum *et al.* 2003). Interestingly, all except one of the O26 strains in this study were found in the EPEC cluster irrespective of whether they were *vtx*-positive or *vtx*-negative. It has been suggested that the division of this serogroup into the pathotypes EPEC and EHEC maybe misleading, as *vtx*-positive strains may arise from *vtx*-negative strains and vice versa (Anjum *et al.* 2003).

Zhang *et al.* (2000) showed that PFGE was a more sensitive typing method than MLEE and RAPD, and demonstrated 'a remarkable heterogeneity among VTEC O26 isolates at the plasmid and chromosomal level'. PFGE has been used in many studies to demonstrate a relationship between outbreak strains (Hiruta *et al.* 2001; McMaster *et al.* 2001; Allerberger *et al.* 2003), to show that strains are not related (Scottish Centre for Infection and Environmental Health 2003), and as an epidemiological tool in farm studies (Pearce *et al.* 2004).

Pearce *et al.* (2004) used serogroup-specific IMS techniques to isolate *E. coli* O26 from a cohort of Scottish beef calves, tested each isolate for the presence of *vtx*1, *vtx*2, *eae* and *ehx* and performed PFGE on all strains to assess the relationship between the strains of VTEC O26 from this cohort. In agreement with Hiruta *et al.* (2001) and Peixoto *et al.* (2001), this data showed that some VTEC O26 isolates have the same PFGE pattern as EPEC O26, suggesting that certain EPEC and VTEC O26 isolates are clonal and the difference between them are the presence or absence of the *vtx* genes.

Conclusions

The increasing incidence of sporadic cases of diarrhoea, HUS and outbreaks involving VTEC O26 in humans and the escalating amount of data on VTEC O26 in cattle, has renewed interest in this *E. coli* serogroup. Recent work on the molecular mechanisms of A/E lesion formation has highlighted significant similarities and differences between VTEC O157 and O26 (Campellone and Leong 2003; Caron *et al.* 2006). Differences in the actin polymerization pathways may confer a competitive advantage to VTEC O26, making it more versatile and able to interact with a wide range of specific hosts and cell types (Ogura *et al.* 2007).

The incidence of VTEC O26 compared with O157 in human disease in a specific geographical location may be more accurately represented by examining the bacterial causes of childhood HUS. In the United Kingdom, the majority of the cases (83%) are attributed to VTEC O157:H7 compared with parts of Europe where this serogroup causes 57% and O26:H11/H- is associated with 15% of the cases (Gerber *et al.* 2002; Lynn *et al.* 2005). These data suggest a true difference in the incidence of VTEC O26 in different geographic locations.

The perceived increase in the incidence of VTEC O26 outbreaks and sporadic infections in humans, and increased prevalence in cattle, may be related to improvements in the methods available to detect *E. coli* O26. Enhanced surveillance in Scotland using a series of phenotypic and genotypic tests for non-O157 contributed to the detection of 10 strains of *E. coli* O26, four of which were VTEC, in 2003 (Locking *et al.* 2004). However, in 2004, none of the seven non-O157 VTEC strains isolated were of serogroup O26 (Locking *et al.* 2006). There has been a shift in the *vtx* genotype of serogroup O26 from *vtx*1 to *vtx*1 and *vtx*2 or *vtx*2 only. It may have occurred because of the emergence of *vtx*2 clonal groups better adapted to the human host or to changes in food consumption (Zhang *et al.* 2000). Alternatively, it might be a result in changes in the prevalence of *E. coli* O26 *vtx*2-positive strains in the animal reservoir. As *vtx*2 is more

commonly associated with severe disease, it is possible that this genotype shift has caused an increase in cases of HUS associated with VTEC O26.

In the United Kingdom, few diagnostic laboratories use molecular methods to detect VTEC and so it is difficult to assess changes in the role of VTEC O26 in diarrhoeal disease. However, improvements in the methods available to detect VTEC O26 and a more standardized approach worldwide will help clarify the true incidence of VTEC O26 (Louie *et al.* 1998).

The epidemiology and shedding patterns of VTEC O26 has been described in detail in studies carried out in Scotland between 2000 and 2005 (Pearce *et al.* 2004; Shaw *et al.* 2004) and mathematical models to predict the transmission have been proposed (Lui *et al.* 2005). VTEC O26 is frequently found in the cattle population but more rarely in food. However, the small number of outbreaks analysed to date are thought to be food-borne rather than associated with direct or indirect contact with livestock or their faeces (Hiruta *et al.* 2001; Allerberger *et al.* 2003). Future work involving molecular detection techniques, such as PCR, and epidemiological methods, such as PFGE, will provide a more accurate assessment of the incidence of the disease and enable the investigation of routes of transmission associated with outbreaks and sporadic cases. The role of VTEC O26 in causing diarrhoea in calves needs to be clarified (Pearce *et al.* 2004). VTEC O157 does not cause disease in animals, and studies of the different characteristics of these two important serogroups may shed light on their pathogenic mechanisms in both humans and animals.

Future studies on the molecular mechanisms of pathogenicity will unravel the relationship between EPEC O26 and VTEC O26, and other *E. coli* serotypes. These studies will help answer important issues such as the route of transmission of VTEC O26, the importance of genetic transfer of virulence factors and the difference in disease characteristics in both humans and animals between different *E. coli* serotypes.

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