1 Introduction

In the second edition of Foodborne Infections and Intoxications (Riemann and Bryan, 1979), Bryan (1979) devoted about four pages to a discussion of enterotoxigenic and enteroinvasive strains of Escherichia coli as foodborne enteric pathogens. The knowledge of E. coli strains that can cause gastrointestinal illness has changed drastically since 1979, as shown by the fact that an entire chapter of this edition (the third) is devoted to foodborne pathogenic E. coli.

In 1895 Escherich first described E. coli, and subsequently, the organism was recognized as a normal inhabitant of human and animal intestinal tracts (Doyle and Padhye, 1989; Neill et al., 1994). The organism is not a major bowel bacterium and, other than its role in providing a source of vitamins in some animals, the function of E. coli in the ecology and physiology of the bowel is unclear (Sussman, 1985). The pathogenic role of E. coli was obscured by its commensal status, and the ability of the organism to cause disease was not suspected for a number of years (Doyle and Padhye, 1989; Neill et al., 1994). Escherichia coli was recognized as a cause of urinary tract infections in the early 1920s (Bettelheim, 1992), and the cause of infantile gastroenteritis was attributed to strains described as enteropathogenic E. coli in the late 1940s (Levine, 1987).
Human *E. coli* strains can be broadly grouped as commensal strains, extraintestinal pathogenic strains, and intestinal pathogenic strains (Russo and Johnson, 2003). Extraintestinal pathogenic *E. coli* acquired genes conferring the ability to cause disease outside of the gastrointestinal tract, and include strains that cause urinary tract, abdominal, and pelvic infections, in addition to septicemia, meningitis, and endocarditis. *E. coli*, however, can cause infection of surgical wounds, in addition to infections in nearly every organ and anatomical site. Intestinal pathogenic strains have acquired virulence factors conferring the ability to cause gastrointestinal diseases, including enteritis and colitis.

Currently, many *E. coli* strains are known to act as intestinal pathogens, varying in the mechanisms by which they produce diarrhea. The mechanisms include the production of toxins and/or host cell attachment factors, and the invasion of colonic mucosal cells (Table 6.1; Guerrant and Thielman, 1995). Usually, a given infection involves more than one virulence factor. At the present time, at least six different categories of diarrheic *E. coli* are known (Table 6.1). A new category called cell-detaching *E. coli* (CDEC), defined by the ability of the bacteria to cause detachment of tissue culture cells from solid supports and by the production of α-hemolysin, pyelonephritis-associated pili and cytotoxic necrotizing factor 1 (CNF-1), has been identified (Fábrega et al., 2002; Okeke et al., 2002). The CDEC may also possess virulence factors found in other categories of *E. coli* (Clarke, 2001; Fábrega et al., 2002; Okeke et al., 2002). *E. coli* strains that induce the formation of actin stress fibers and activation of DNA synthesis in cell cultures, leading to the formation of multi-nucleated giant cells, have been referred to as necrotoxigenic *E. coli* (NTEC). There are two toxins involved, CNF-1 in NTEC1 strains, and cytotoxic necrotizing factor-2, CNF-2, in NTEC2 strains. Many NTEC also produce cytolethal distending toxins (De Rycke et al., 1999; Mainil et al., 2003). Interestingly, the gene that encodes CNF-1 in NTEC1 strains is found on a pathogenicity island that also has genes that

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encode for α-hemolysin and P-fimbriae (De Rycke et al., 1999); therefore, NTEC1 may be related to CDEC. Due to the promiscuity of genetic transfer in Gram-negative organisms (Clarke, 2001), it is probable that additional diarrheic E. coli strains will emerge. The characteristics of the six major categories of diarrheagenic E. coli are discussed below.

2 Characteristics of Escherichia coli

2.1 Classification and biochemical characteristics

Escherichia coli is in the family Enterobacteriaceae. The organism is a Gram-negative, non-spore-forming, straight rod (1.1–1.5 μm × 2.0–6.0 μm) arranged in pairs or singly; is motile by means of peritrichous flagella or may be non-motile; and may have capsules or microcapsules. Escherichia coli is a facultatively anaerobic, chemo-organotrophic microorganism with an optimum growth temperature of 37 °C (Brenner, 1984; Ørskov, 1984). It is oxidase negative, catalase positive, fermentative (glucose, lactose, D-mannitol, D-sorbitol, arabinose, maltose), reduces nitrate, and is β-galactosidase positive. Approximately 95 % of E. coli strains are indole and methyl red positive, but are Voges-Proskauer and citrate negative (Doyle and Padhye, 1989). Interestingly, DNA relatedness indicates that E. coli and Shigella form a single species, and it is difficult to separate the two biochemically (Brenner, 1984). Shigella strains were placed in a different genus in the 1940s to distinguish them from non-pathogenic E. coli; however, a number of studies based on genetic typing and DNA sequencing have shown that Shigella strains fall within E. coli (Lan and Reeves, 2002). The separate nomenclature is maintained for medical and epidemiological purposes.

Serotyping and serogrouping of E. coli is useful for subdividing the species into serovars. Serological typing in E. coli involves serological identification of three surface antigens: O (somatic lipopolysaccharide), K (capsular) and H (flagellar). Although the numbers of the different E. coli O, K, and H antigens reported in the literature vary, Ørskov and Ørskov (1992) state that there are 173 O antigens, 80 K antigens and 56 H antigens. Mol and Oudega (1996) have suggested that the fimbrial (F) surface antigens should be a fourth component of serological testing. Determining the serogroup (O antigen) and serotype (O and H, and often K antigens) is an important means of defining the various pathogenic strains of E. coli, since certain serotypes are associated with the various categories of diarrheagenic E. coli. For example, E. coli serotype O157:H7 is an enterohemorrhagic E. coli, and E. coli serogroup O124 is an enteroinvasive strain. E. coli serotyping is important for making the proper diagnosis and for performing foodborne outbreak and epidemiological investigations. However, novel E. coli serotypes frequently emerge as intestinal pathogens and, when recognized, are included in a specific category of diarrheagenic E. coli. Thus serotyping alone cannot be relied on for categorizing a strain of E. coli, and the identification of specific virulence characteristics/genes must also be performed (Barlow et al., 1999).
2.2 **Escherichia coli** in foods: growth and survival

Since *E. coli* is an inhabitant of the gastrointestinal tract of humans and animals, it is expected to be present in the environment, water and food. The organism can be present in the environment due to animal defecation or contamination with untreated human sewage, in foods of animal origin such as meat or milk, in produce from manured land or from plots irrigated with fecally-contaminated water, in cooked or uncooked foods prepared by infected food handlers, and in water contaminated with human sewage or animal waste.

*E. coli* strains do not grow under refrigeration conditions; however, the organism can survive for weeks at 4°C or −20°C. The limits of temperature for growth of *E. coli* are 7–46°C, and the optimum growth temperature is approximately 37°C (Bell and Kyriakides, 1998). The heat resistance of *E. coli* is similar to that of other enteric bacteria; however, heat sensitivity is affected by the food environment and exposure of the organism to prior stress or growth conditions. *E. coli* generally grows within the pH range of 4.4–9.0, at an aw of at least 0.95, and at NaCl levels of less than 8.5 % (Bell and Kyriakides, 1998). Many studies related to the growth and survival of *E. coli* have been performed with *E. coli* O157:H7. These are discussed in Section 8.

2.3 **Escherichia coli** as an indicator organism

As a normal member of the gastrointestinal tract of humans and animals, the presence of *E. coli* in the environment, water or food suggests fecal contamination. The presence of *E. coli* in a food implies that enteric pathogens may also be present. However, studies have shown that the presence or absence of fecal pathogens cannot be correlated with detection of *E. coli* (Pierson and Smoot, 2001). The presence of the organism in heat-processed foods may represent process failure, post-processing contamination from equipment or personnel, or contact with raw product (Pierson and Smoot, 2001). At best, the presence of *E. coli* in food or water is an indication of uncleanliness and careless handling.

3 **Enteroinvasive Escherichia coli** (EIEC)

The enteroinvasive *E. coli* strains are a cause of bacillary dysentery. These strains are biochemically and genetically related to *Shigella*, cause disease symptoms similar to those of *Shigella*, and have *Shigella*-like characteristics such as the lack of motility, the inability to ferment lactose and failure to decarboxylate lysine. The EIEC are able to invade HeLa cells and to induce keratoconjunctivitis in the guinea pig eye (Sérény test) (Hale et al., 1997; Nataro and Kaper, 1998). In most patients an EIEC infection results in watery diarrhea; however, in a few cases stools may contain blood and mucus. The infective dose of EIEC is several logs higher than that of *Shigella* (Hale et al., 1997),...
indicating that person-to-person spread is uncommon, although it has been reported (Harris et al., 1985).

The site of EIEC infection is the colonic mucosa. The bacterial cells attach to the epithelial cells of the colon with subsequent penetration of the enterocytes via endocytosis. The endocytic vacuole is lysed followed by intracellular multiplication of the bacterial cells. There is directional movement of EIEC through the cytoplasm mediated by the attachment of cellular actin to one pole of the bacterial cell; actin aids in propelling the bacteria into adjacent epithelial cells (Nataro and Kaper, 1998). Thus there is cell-to-cell spread of EIEC without entrance into the extracellular milieu.

A 140-MDa plasmid (pINV) encodes the genes necessary for EIEC to invade, multiply and survive within the colonic enterocytes. The *ipa* (invasion plasmid antigen) genes present on the plasmid encode the Ipa proteins, IpaA–IpaD, which are necessary for the invasive phenotype (Hale et al., 1997; Nataro and Kaper, 1998). The EIEC plasmid has virulence genes identical to those present on the *Shigella* 120–140-MDa invasion plasmid. The sequences of three genes, *ipgD*, *mxiC* and *mxiA*, in the invasion region of the virulence plasmid of *Shigella* and EIEC were analyzed to determine the evolutionary relationships of the pINV plasmids (Lan et al., 2001). Two distinct forms of the plasmid were identified in *Shigella* species, pINV A and pINV B, and the EIEC strains had plasmids identical to those found in *Shigella* strains. Furthermore, Wang et al. (2001a) found that 12 of the 33 O-antigen forms in *Shigella* were identical to those of *E. coli* strains.

Food- and waterborne outbreaks of EIEC have occurred; however, they are not common in industrialized nations. Implicated foods include French soft cheeses, potato salad, and guacamole (Gordillo et al., 1992; Hale et al., 1997; Willshaw et al., 2000). Suspect foods are generally cooked foods that are not reheated after being handled by infected food workers, but raw foods may also be involved. There has been one report of an outbreak in an institution due to person-to-person transfer of EIEC (Harris et al., 1985). Traveler’s diarrhea has also been associated with EIEC infection (Hale et al., 1997). Infections by EIEC do not appear to be important contributors to morbidity in developed countries; such infections are probably more important in developing countries, particularly among young children. Similar to *Shigella*, the reservoir for EIEC is the human intestinal tract.

A virulence antigen-specific, monoclonal antibody-based, enzyme-linked immunoassay has been used to detect *Shigella* and EIEC (Pal et al., 1997). In addition, DNA probes and primers have been developed and used to detect *Shigella* and EIEC by hybridization or by the polymerase chain reaction (PCR) respectively (Houng et al., 1997). López-Saucedo et al. (2003) described a multiplex PCR that differentiated enterotoxigenic *E. coli*, enteropathogenic *E. coli*, Shiga toxin-producing *E. coli* and EIEC in stool samples. A PCR assay using enriched stool samples from children with acute diarrhea was more sensitive than stool culture or colony hybridization for detection of *Shigella* and EIEC (Dutta et al., 2001). While it is possible to differentiate between the various diarrheagenic *E. coli* strains via multiplex PCR, it is difficult to differentiate between *Shigella* and EIEC.
4 Diffusely adherent *Escherichia coli* (DAEC)

The category known as diffusely adherent *E. coli* (DAEC) is poorly characterized, and the involvement of DAEC in diarrhea remains controversial. When bacteria attach uniformly to the surface of HeLa or HEp-2 cells the adherence is termed diffuse; whereas in localized adherence the bacteria adhere in groups at one or a few sites on the cell surface (Scaletsky *et al.*, 1984). Bilge *et al.* (1989) designated a fimbrial adhesin, F1845, as responsible for diffuse HEp-2 cell adhesion by diarrheic *E. coli* isolates. A DNA probe targeting the *daaC* gene, associated with expression of the F1845 fimbriae, has been developed to detect DAEC (Bilge *et al.*, 1989). The *daaC* gene can be found either on the bacterial chromosome or on a plasmid. The probe is specific for this gene; however, it is rather insensitive, which suggests that other adhesins are also responsible for the diffuse-adherent pattern (Willshaw *et al.*, 2000).

A second putative adhesin that mediates the diffuse adherence phenotype, designated AIDA-I (adhesin involved in diffuse adherence), is a 100-kDa cell surface protein (Benz and Schmidt, 1992). Some strains of DAEC induce finger-like projections, which jut from the surface of epithelial cells (HEp-2 and Caco-2 cells). These projections enclose the bacterial cells, embedding and protecting them from gentamicin; however, the bacteria are not intracellular (Cookson and Nataro, 1996). The role of these finger-like projections in pathogenesis is unknown.

Some strains of DAEC are not diarrheic; however, if infected with a diarrheic strain the patient has fever and vomiting, and stools are watery and mucoid. Diarrhea caused by DAEC occurs in developing countries, mainly in children between 48 and 60 months of age; infants are rarely affected (Cookson and Nataro, 1996; Nataro and Kaper, 1998). Nursing infants may be protected against DAEC because human milk proteins have been shown to inhibit the adherence of DAEC (Nascimento de Araújo and Giugliano, 2000). Jallat *et al.* (1993) demonstrated that most of the *E. coli* strains isolated from stools of diarrheic infants, children and adults in a French hospital were DAEC (100/262 were diffusely adherent on HEp-2 cells). Only one-third of the DAEC strains hybridized with the F1845 *daaC* probe, however, indicating that DAEC strains are quite heterogeneous. In a study of 24 diarrheic children, Poitrineau *et al.* (1995) found that vomiting but not diarrhea was significantly associated with the presence of DAEC in their stools. Those children carrying F1845 DNA probe-positive DAEC had approximately three times longer hospital stays than children harboring other DAEC types. Strains in the DAEC category apparently vary in the level of pathogenicity.

5 Enteroaggregative *Escherichia coli* (EAEC)

*Escherichia coli* strains that do not secrete labile toxin, stable toxin or Shiga toxin and adhere in an aggregative or ‘stacked brick’ (AA phenotype) adhesion pattern to HEp-2 cells are known as enteroaggregative *E. coli* (EAEC) (Nataro *et al.*, 1995; Law and Chart, 1998). This definition may include both pathogenic and non-pathogenic strains. A number of diarrheic outbreaks have been caused by EAEC and associated
with food or drinking water; however, the organism was seldom isolated from the suspect vehicle (Cobeljic et al., 1996; Itoh et al., 1997; Smith et al., 1997; Nataro et al., 1998; Okeke and Nataro, 2001). Strains of EAEC have been isolated from foods such as formula from baby feeding bottles (Morais et al., 1997) and tabletop sauces such as guacamole from Mexican-style restaurants (Adachi et al., 2002a).

EAEC strains are a common cause of persistent diarrhea in children in developing countries; however, disease caused by EAEC is probably underreported and underdiagnosed as a cause of childhood diarrhea in industrialized countries (Okeke and Nataro, 2001). Protein components of human milk inhibit the adhesion of EAEC to HeLa cells (Nascimento de Araújo and Giugliano, 2000). It is likely that infants are protected against EAEC diarrhea while they are nursing. In adults, EAEC has been reported as a causative agent of diarrhea in individuals who travel to developing countries (Adachi et al., 2001, 2002b; Okeke and Nataro, 2001) and in HIV-infected individuals (Okeke and Nataro, 2001). Other immunocompromised populations are also probably susceptible to EAEC-induced diarrhea.

The diarrhea induced by EAEC is watery and often protracted, and is associated with abdominal pain. Borborygmus (rumbling due to gas), low-grade fever, vomiting and dehydration may occur. Gross mucus and blood may be present in the stools, and up to one-third of patients may have grossly bloody stools (Nataro et al., 1998; Okeke and Nataro, 2001). Histologically, a thick mucous gel is present on the intestinal mucosa, and there are necrotic lesions in the ileal mucosa (Eslava et al., 1998). The inflammatory cytokines IL-8 and IL-1, produced during EAEC infection, induce mucosal inflammation (Okeke and Nataro, 2001; Greenberg et al., 2002). Infection with EAEC may also be asymptomatic. EAEC infections may lead to malnutrition and growth retardation in infants and children (Nataro et al., 1998; Steiner et al., 1998). Oral hydration is an effective therapy (Law and Chart, 1998; Nataro et al., 1998; Smith and Cheasty, 1998).

A three-stage model has been proposed for EAEC pathogenesis: Stage I involves initial adherence to the intestinal mucosa and mucous layer; stage II involves enhanced production of mucus, leading to a thick EAEC-encrusted biofilm on the mucosal surface; and stage III involves elaboration of cytotoxin(s), which result in intestinal secretion and damage to the intestinal mucosa (Nataro et al., 1998; Okeke and Nataro, 2001). The thick cover on the mucosal surface may promote tenacious colonization and lead to malnutrition. The persistent diarrhea seen in EAEC-infected patients may be due to the inability of individuals with pathogen-induced malnutrition to repair the damage done to the intestinal mucosa. Pathogenicity of EAEC has been modeled using tissue culture (Nataro et al., 1996) and gnotobiotic piglets (Tzipori et al., 1992).

5.1 Virulence factors

Information concerning virulence factors in EAEC is limited and confusing. There appear to be several types of fimbriae involved with aggregative attachment, and while the pathology suggests that a toxin is involved in EAEC diarrhea, it is not clear that the toxin(s) responsible have been identified. A study of iron utilization has
shown that EAEC are able to utilize heme or hemoglobin as the sole iron source and produce siderophores at a level similar to that of *Shigella* and enterohemorrhagic *E. coli* (Okeke *et al*., 2004). Most strains possessed genes associated with multiple iron utilization systems, which may provide EAEC with a competitive advantage over other bacteria that are negative for these systems. Analysis of EAEC strains isolated from Mongolian children with diarrhea has shown that AggR (transcriptional activator)-positive strains that caused diarrhea were more likely to possess several other EAEC virulence genes than AggR-negative strains. Furthermore, the isolation of AggR-positive EAEC was significantly higher in the diarrheal group than in controls; thus, AggR may serve as a marker for virulent EAEC strains (Sarantuya *et al*., 2004).

5.1.1 Attachment
Using biopsies from normal patients, Knutton *et al*. (1992) demonstrated attachment of EAEC to the colonic (44/44 biopsy samples) and the ileal mucosa (36/44 of biopsy samples). None of the EAEC strains attached to the duodenal mucosa. Knutton *et al*. (1992) suggested that EAEC is a large-bowel pathogen that colonizes the colon by adhesion mediated by fimbriae. A number of EAEC adherence factors have been demonstrated. Nataro *et al*. (1992) described aggregative adherence fimbriae I (AAF/I), and Czeczulin *et al*. (1997) characterized AAF/II. However, by using DNA probes for AAF/I and AAF/II, Czeczulin *et al*. (1997) found that only a minority of EAEC strains possessed these fimbriae. Other aggregative fimbriae have been described by Knutton *et al*. (1992) and Collinson *et al*. (1992). In addition, afimbrial adhesins are expressed by some strains of EAEC (Okeke and Nataro, 2001).

5.1.2 Putative toxins
The EAST1 toxin, a plasmid-mediated, low molecular weight, heat-stable toxin with an *in vitro* mode of action similar to that of the ETEC heat-stable toxin, was first demonstrated in EAEC (Savarino *et al*., 1991). The astA gene encoding EAST1 was found in approximately 41 % of EAEC strains (Okeke and Nataro, 2001). It is not clear, however, that EAST1 has a role in EAEC-induced diarrhea *in vivo* (Navarro-Garcia *et al*., 1998). A more detailed discussion of EAST1 can be found in Section 6.

A high molecular weight (108-kDa), heat-labile protein toxin was found in EAEC (Navarro-Garcia *et al*., 1998). A partially purified preparation induced tissue damage, inflammation and secretion of mucus in isolated rat jejunum. Eslava *et al*. (1998) reported on the genetic cloning, sequencing and characterization of the 108-kDa toxin. The toxin gene (*pet*) is located on the 65-MDa EAEC virulence plasmid, which also contains the genes for the aggregative phenotype, AA. The plasmid-encoded toxin (Pet) appears to belong to the autotransporter class of secreted proteins, and is highly homologous to other autotransporter proteins such as the EspP protease of EHEC and the cryptic protein EspC of enteropathogenic *E. coli* (Eslava *et al*., 1998). Okeke and Nataro (2001) reported that 18–44 % of EAEC isolates possess Pet. Morabito *et al*. (1998) isolated EAEC strains that were involved in an outbreak of HUS. These strains were unusual in that they produced Stx2, had the AA phenotype, and possessed the astA gene for EAST1 but lacked the EHEC genes, *eaeA*, *hly* and *katP*. 
5.2 Heterogeneity of EAEC strains

EAEC form a very heterogeneous group comprising more than fifty O serogroups (Chart et al., 1997). Serotyping is not useful for identifying EAEC strains. Types of fimbriae vary from bundles of fine filaments (Knutton et al., 1992) and thin fimbriae (Collinson et al., 1992) to bundle forming fimbriae (Nataro et al., 1992; Czeczulin et al., 1997); however, not all EAEC express fimbriae (Chart et al., 1997). In addition, not all strains of EAEC produce the EAST1 or Pet toxins (Savarino et al., 1996; Eslava et al., 1998). Nataro et al. (1995) also demonstrated that EAEC strains are heterogeneous in their ability to induce diarrhea in adult volunteers. The heterogeneity of EAEC renders identification of strains and diagnosis of EAEC-induced illnesses difficult.

5.3 Diagnosis of EAEC infections

The most definitive identification of EAEC is the demonstration of adhesion to HEp-2 cells (Law and Chart, 1998; Miqdady et al., 2002). However, the technique is only suitable for use in research laboratories. It is time-consuming and cumbersome, and the type of adherence can be easily misinterpreted.

Utilizing the adhesion-associated region of the adherence plasmid of EAEC, Baudry et al. (1990) and DebRoy et al. (1994) developed DNA probes for the identification of EAEC strains. However, neither probe identified all EAEC having the AA phenotype. The probe developed by Baudry's group detected 70.5 % (43/61) of isolates, whereas the probe developed by DebRoy and coworkers detected 93.4 % (57/61).

Schmidt et al. (1995) developed a PCR assay based on the probe of Baudry et al. (1990). Of 50 EAEC strains (positive in the HEp-2 adherence assay), 88 % (44/50) were positive with the Baudry probe, and 86 % (43/50) were positive using the PCR. Both probes reacted with less than 1 % (4/418) of other E. coli strains tested (Schmidt et al., 1995). Thus the probes and PCR tests were quite specific but did not detect all EAEC that showed the typical AA phenotype. Therefore, the HEp-2 adherence assay is the only reliable method for identification of EAEC strains; however, the test does not distinguish pathogenic from non-pathogenic strains of EAEC.

A multiplex PCR using three plasmid-borne genes (aggregative adherence (AA) probe, aap and aggR) was tested on 28 AA-positive E. coli isolated from diarrheic patients and detected 23/28 (82 %) of the strains (Cerna et al., 2003). It appears that the only reliable technique for detection of EAEC is the determination of the AA phenotype using a tissue culture assay.

Clearly, additional studies on EAEC and the determination of the type of adherence factors involved in the aggregative type of adherence are needed. It is probable that the aggregative pattern is mediated by different types of adhesion molecules. The literature indicates that not all EAEC strains cause diarrhea. Is the aggregative pattern important for virulence in vivo, or is it merely a diagnostic tool to detect a certain type of E. coli? Thus, tests to identify pathogenic EAEC and differentiate diarrheic from non-diarrheic strains of EAEC are needed. The role of the EAST1 and Pet toxins as in vivo agents of pathogenesis in EAEC strains is uncertain. Generation of
mutants lacking the expression of these toxins or cloning of EAEC virulence factors into laboratory strains of *E. coli* and use in cell culture assays, animal models (Law and Chart, 1998) and human volunteers may be useful in understanding the virulence of EAEC. Growth retardation and other growth deficits appear to be related to EAEC infections (Nataro *et al*., 1998; Steiner *et al*., 1998). Does malnutrition predispose to EAEC infection? Does EAEC-induced mucosal damage lead to malabsorption of nutrients? Does EAEC-induced stimulation of mucus formation impose a barrier to intestinal absorption of nutrients? Animal studies should clarify the putative role of EAEC in malnutrition.

6 Enterotoxigenic *Escherichia coli* (ETEC)

6.1 The disease

In developed countries ETEC strains are an uncommon cause of diarrhea; however, in developing countries they are a major cause of diarrhea, with high morbidity and mortality in infants, young children and the elderly. In addition, the organism is the primary cause of traveler’s diarrhea in visitors to developing countries (Cohen and Giannella, 1995; O’Brien and Holmes, 1996).

Newborn and young domestic animals (calves, lambs, and pigs) are susceptible to ETEC-induced diarrhea; however, ETEC do not cause disease in adult animals (Gyles, 1992). The ETEC colonize the small intestine of both human adults and children by attaching to the enterocytic brush border with the aid of bacterial adherence factors; however, the organisms do not invade or damage intestinal cells (Cohen and Gianella, 1995). The ETEC secrete toxin(s), which lead to the production of a non-inflammatory watery diarrhea. Blood, mucus, and leukocytes are absent in stools. The infected individuals may show nausea and mild to moderate abdominal cramping, but without fever (Neill *et al*., 1994; Cohen and Gianella, 1995). Diarrhea may be prolonged in children, leading to severe dehydration, and mortality can be high. Serious malnutrition may result in children infected with ETEC. Traveler’s diarrhea in adults is usually a mild self-limited illness lasting 1–5 days (Neill *et al*., 1994; Cohen and Gianella, 1995).

ETEC infection is transmitted through the ingestion of contaminated food or water. An ETEC-infected food handler with poor personal hygiene can contaminate food and water. Also, ETEC-containing sewage can contaminate potable water (Black *et al*., 1981). Studies with human volunteers indicate that the infective dose is approximately $10^8$ organisms (Levine *et al*., 1977), therefore person-to-person transmission of ETEC infection is not likely under most circumstances. Humans appear to be the major reservoir for ETEC, and there are no animal reservoirs, although young animals are susceptible to ETEC infection (Doyle and Padhye, 1989).

Antibiotic treatment is generally not recommended for most cases of traveler’s diarrhea, since antibiotic use may lead to antibiotic resistance in ETEC and may also change the intestinal flora. If the diarrhea is severe or prolonged, the infection can be treated with trimethoprim/sulfmethoxazole, and rehydration therapy may be
required. Mild cases of ETEC-induced traveler’s diarrhea can be treated with antidiarrheal drugs to limit fluid accumulation and intestinal mobility (Berkow, 1992; Cohen and Giannella, 1995).

The best prophylaxis against ETEC infection and other gastrointestinal diseases in infants is breastfeeding (Black and Lanata, 1995; Pickering et al., 1995). In a study involving Bangladeshi children, Clemens et al. (1997) found that exclusive breastfeeding of infants (children less than 1 year of age) was protective against severe ETEC-induced illness. This protective effect was not observed with breastfed children during the second and third years of life. Breastfeeding provided significantly greater protection against diarrhea induced by *Vibrio cholerae* as compared to ETEC-induced diarrhea (Clemens et al., 1997). In ETEC-infected infants and children, oral rehydration is an effective therapy, and rehydration prior to the occurrence of severe diarrheic dehydration can be lifesaving. Antimotility agents and antibiotic therapy are not recommended for use in ETEC-infected children. In developing countries, antibiotic resistance is common, and the use of antibiotics often does not remove the offending organisms from the gut (Pickering et al., 1995). Similarly, antimotility agents interfere with peristaltic removal of the pathogen. Vaccines for use in humans to control ETEC infections are not available (O’Brien and Holmes, 1996).

### 6.2 Foodborne outbreaks

Water and food have been implicated in outbreaks of ETEC (CDC, 1994; Table 6.2). Foods implicated in outbreaks include salads, dipping sauces and ready-to-eat items, including hot dogs, cold roast beef, cold turkey, and Brie cheese (foods that are served raw or foods that are cooked but served cold). Although ETEC does not appear to be a major cause of diarrheic foodborne outbreaks in the United States, many disease cases may go unrecognized due to the non-availability of laboratory tests for identification of ETEC strains. Beatty et al. (2004) reported results of an 8-year study on the incidence of outbreaks due to ETEC in the US and on cruise ships. Sixteen outbreaks due to ETEC occurred from 1996 to 2003, and *E. coli* O169:H41 was the serotype identified in 10 of the outbreaks. The vehicle of infection was identified in 11 of the outbreaks, and included drinking water, ice, various vegetables and salads, enchiladas, tacos, tortilla chips, quesadillas, fajitas, chicken, lasagna, and catfish.

### 6.3 Basis of pathogenicity

The ETEC cause diarrheal illness by adherence and colonization of the intestinal mucosa, and the synthesis and release of at least one member of two groups of enterotoxins – heat-labile toxins (LT) and heat-stable toxins (ST) (Cohen and Giannella, 1995). ETEC strains may produce an LT only or an ST only, or both an LT and an ST.

#### 6.3.1 The LT enterotoxins

Some features of the LT enterotoxins of ETEC are listed in Table 6.3. Polyclonal antibodies raised against a particular LT-I toxin neutralize other LT-I s and cholera toxin (CT) produced by *V. cholerae*, but do not neutralize the activity of LT-II toxins.
### Table 6.2  Foodborne outbreaks in which ETEC strains were implicated

<table>
<thead>
<tr>
<th>Reference</th>
<th>Toxin</th>
<th>Number ill</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryder <em>et al</em>., 1976</td>
<td>ST</td>
<td>55</td>
<td>Outbreak occurred in a children’s hospital in the United States; baby formula was implicated</td>
</tr>
<tr>
<td>Merson <em>et al</em>., 1976</td>
<td>ST/LT</td>
<td>8</td>
<td>Outbreak occurred in travelers attending a meeting in Mexico City; salads containing raw vegetables were implicated</td>
</tr>
<tr>
<td></td>
<td>LT</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Hobbs <em>et al</em>., 1976</td>
<td>ST</td>
<td>67</td>
<td>Outbreak occurred on a cruise ship; food suspected</td>
</tr>
<tr>
<td>Kudoh <em>et al</em>., 1977</td>
<td>ST</td>
<td>129</td>
<td>Two separate outbreaks in two different locations in Japan (food eaten not stated); outbreaks associated with eating lunch</td>
</tr>
<tr>
<td>Danielsson <em>et al</em>., 1979</td>
<td>LT</td>
<td>60</td>
<td>Outbreak occurred in a Swedish restaurant; shrimp and mushroom salads were implicated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>2</td>
<td>Incident occurred in a Swedish home; cold, shop-sliced roast beef implicated</td>
</tr>
<tr>
<td>Lumish <em>et al</em>., 1980</td>
<td>LT</td>
<td>349</td>
<td>Outbreak occurred on two separate trips of the same cruise ship; drinking water and crabmeat cocktail were implicated</td>
</tr>
<tr>
<td>Taylor <em>et al</em>., 1982</td>
<td>ST/LT</td>
<td>415</td>
<td>Outbreak occurred in a restaurant in Wisconsin; Mexican food was implicated (food items included sauces, garnish, flour tortillas, and guacamole)</td>
</tr>
<tr>
<td>Wood <em>et al</em>., 1983</td>
<td>LT</td>
<td>282</td>
<td>Outbreak occurred in a hospital in Texas; associated with eating in hospital cafeteria; no specific food was implicated</td>
</tr>
<tr>
<td>Riordan <em>et al</em>., 1985</td>
<td>ST/LT</td>
<td>27</td>
<td>Outbreak occurred at a cold buffet at a school in England; curried turkey mayonnaise was implicated</td>
</tr>
<tr>
<td>MacDonald <em>et al</em>., 1985</td>
<td>ST</td>
<td>45</td>
<td>Clusters of outbreaks occurred at office parties in Washington, DC; French Brie cheese was implicated; cheese from the same plant (same brand and lot) was implicated in outbreaks in Illinois (75 cases), Wisconsin (35 cases), Georgia (10 cases) and Colorado (4 cases). The same brand of cheese caused outbreaks in Denmark, the Netherlands and Sweden</td>
</tr>
<tr>
<td>CDC, 1994</td>
<td>LT, ST</td>
<td>47</td>
<td>Outbreak occurred on a plane from North Carolina to Rhode Island; garden salad was implicated</td>
</tr>
<tr>
<td></td>
<td>LT, ST</td>
<td>97</td>
<td>Outbreak occurred at a buffet served in a mountain lodge in New Hampshire; tabouleh salad was implicated</td>
</tr>
<tr>
<td>Mitsuda <em>et al</em>., 1998</td>
<td>ST1b</td>
<td>&gt;600</td>
<td>Outbreak occurred with school lunches at four elementary schools in Japan; tuna paste implicated – raw carrots, onions and cucumbers that were part of the tuna paste were probably contaminated with ETEC</td>
</tr>
</tbody>
</table>
**Table 6.2** Foodborne outbreaks in which ETEC strains were implicated—cont’d

<table>
<thead>
<tr>
<th>Reference</th>
<th>Toxin</th>
<th>Number ill</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roels et al., 1998</td>
<td>ST</td>
<td>372–645</td>
<td>Outbreak occurred at a banquet in Milwaukee, Wisconsin; pan-fried spiced potatoes were implicated</td>
</tr>
<tr>
<td></td>
<td>ST and LT/ST</td>
<td>97</td>
<td>Outbreak occurred on a cruise ship; drinking ship’s tap water and/or beverages with ice were implicated</td>
</tr>
<tr>
<td>Daniels et al., 2000</td>
<td>ST, LT and LT/ST</td>
<td>19</td>
<td>Outbreak occurred on a cruise ship; beverages with ice and ice water were implicated</td>
</tr>
<tr>
<td></td>
<td>ST, LT and LT/ST</td>
<td>197</td>
<td>Outbreak occurred on a cruise ship; bottled water and beverages with ice were implicated</td>
</tr>
<tr>
<td>Huerta et al., 2000</td>
<td>ST, LT and LT/ST</td>
<td>229</td>
<td>Outbreak occurred at military posts and civilian communities in the Golan Heights, Israel; drinking water was implicated</td>
</tr>
<tr>
<td>Naimi et al., 2003</td>
<td>ST, LT and LT/ST</td>
<td>77</td>
<td>Outbreak occurred in a restaurant in Minnesota; parsley from Mexico was implicated</td>
</tr>
</tbody>
</table>

Modified from Fratamico et al. (2002).

**Table 6.3** Toxins of enterotoxigenic *E. coli*

<table>
<thead>
<tr>
<th>Toxin vs heat</th>
<th>Variants</th>
<th>Preferred receptor</th>
<th>Structure</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat-labile (LT)</td>
<td>LTh-I, LTP-I</td>
<td>GM1</td>
<td>One A unit (~240 amino acids) combined with five B units (100 amino acids each)</td>
<td>Activation of adenylate cyclase with stimulation of intestinal CI– secretion</td>
</tr>
<tr>
<td>Heat-stable (ST)</td>
<td>LT-IIa, LT-IIb, STIa (STp)</td>
<td>GD1b, GD1b, Guanylate cyclase STαR (on human enzyme)</td>
<td>18-amino acid peptide</td>
<td>Activation of guanylate cyclase with stimulation of CI– secretion</td>
</tr>
<tr>
<td></td>
<td>STIb (STh)</td>
<td>STIb (STh), Guanylate cyclase STαR (on human enzyme)</td>
<td>19-amino acid peptide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>STII</td>
<td>Unknown</td>
<td>48-amino acid peptide</td>
<td>Mode of action unknown; stimulation of intestinal HCO₃⁻ secretion</td>
</tr>
</tbody>
</table>

Modified from O’Brien and Holmes (1996)

* After 30 minutes at 100 °C, LT toxin activity is lost; ST toxin activity is retained.
Thus, there is a close relationship between CT and LT-I. Antibodies to a particular LT-II neutralize LT-IIs but not LT-I or CT (O’Brien and Holmes, 1996). The LTh-I variant is of human origin, whereas, the LTp-I is of porcine origin. The CT-encoding genes are carried on the genome of a filamentous bacteriophage maintained as an integrated prophage (Davis and Waldor, 2003); however, the genes for LT-I are plasmid-mediated. The genes for LT-II are chromosomally encoded (O’Brien and Holmes, 1996). LT-I causes a milder disease in humans than CT; however, LT-II does not appear to be involved in human disease and is found primarily in animal isolates (O’Brien and Holmes, 1996).

Similar to CT, the LTs are oligomeric peptides consisting of one A polypeptide subunit non-covalently bound to five B polypeptides. The A subunits of LT-I and LT-II have approximately 50 % amino acid identity; whereas, the B subunits of LT-I and LT-II are approximately 10 % identical (O’Brien and Holmes, 1996). The B subunits of LT-I are ring structures that bind to the ganglioside GM1 on the host cell. The A subunit undergoes proteolytic nicking to produce A1 and A2 fragments. The A1 fragment is linked to A2 by a disulfide bond, and the A2 fragment is bound to the B subunits. After binding to the host cell, the toxin is endocytosed and translocated through the cell (Butterton and Calderwood, 1995; Nataro and Kaper, 1998). The cellular target of LT is adenylate cyclase located on the membrane of intestinal epithelial cells. The A1 fragment exhibits ADP-ribosyl transferase activity. ADP ribosylation of a GTP-binding protein mediates activation of adenylate cyclase, with a resultant increase in cyclic AMP within the intestinal mucosa. The net result is the stimulation of chloride secretion and a decrease in sodium absorption. The increased luminal ion content leads to a loss of fluid and electrolytes with production of a watery diarrhea (Butterton and Calderwood, 1995; Nataro and Kaper, 1998).

For CT activity proteolytic nicking of the A subunit is necessary (Butterton and Calderwood, 1995), whereas nicking is not necessary for enzymatic activity of the LT-I A subunit (Grant et al., 1994). Nicking does, however, enhance the biological and enzymatic activity of LT. Tsuji et al. (1997) constructed an ETEC mutant in which the nicking region of the A-subunit of LT was deleted. The mutant had less diarrheal activity with decreased induction of cyclic AMP; thus, nicking of the A-subunit of LT-I appears to be necessary for optimum activity of the toxin. Other bacteria that produce LT-like toxins include Klebsiella, Enterobacter, Aeromonas, Plesiomonas, Campylobacter and Salmonella (Cohen and Gianella, 1995).

6.3.2 The ST enterotoxins
Certain characteristics of the ST enterotoxins are shown in Table 6.3. These monomeric toxins are subdivided into STI (STa) and STII (STb) families. Two toxins are found in the STI family (Table 6.3), including one of porcine origin – STp, an 18-amino-acid peptide. The other, STh, is of human origin and consists of 19 amino acids. Three intramolecular disulfide bonds are present in the STI peptides. STI molecules are heat and acid stable, are not denatured by detergents, are water and methanol soluble, and are resistant to proteases. With disruption of the disulfide bonds, the toxins become inactive (Cohen and Gianella, 1995; O’Brien and Holmes,
The STI peptides are poorly antigenic, and must be conjugated to a carrier protein to prepare antisera for diagnostic purposes. Genes for both STI and STII are located predominately on plasmids, but some are on transposable elements. Genes encoding LT, ST, colonization factors, colicin and antibiotic resistance may be present on the same plasmid (Cohen and Gianella, 1995; O’Brien and Holmes, 1996; Nataro and Kaper, 1998).

STI is synthesized in the bacterial cell cytoplasm as a precursor protein consisting of a PRE-region (amino acid residues 1–19), a PRO-region (amino acid residues 20–54) and a MATURE-region (amino acid residues 55–72). The precursor protein is translocated across the inner membrane into the periplasmic space via Sec proteins of the type II secretion pathway. The PRE-region acts as a signal protein in the translocation process and is cleaved during or after translocation. In the periplasmic space, the protein consists of the PRO- and MATURE-regions (Okamoto and Takahara, 1990). The PRO-region is then cleaved, disulfide bonds are formed in the mature 18-amino acid toxin, and the toxin is translocated across the outer membrane in an unknown manner (Yamanaka et al., 1994, 1997). Yamanaka et al. (1998) have shown that an outer membrane protein, TolC, is involved in some manner in the translocation of periplasmic STI across the outer membrane into the external environment.

The major receptor for STI is guanylate cyclase C in the membrane of enterocytes in the small intestine. Binding of the enzyme by STI leads to an accumulation of cyclic GMP, and secretion of chloride and water into the intestinal lumen (Cohen and Gianella, 1995; O’Brien and Holmes, 1996; Nataro and Kaper, 1998). Guanylin is a mammalian hormone which aids in the regulation of fluid and electrolyte absorption in the gut. STs and guanylin are homologous and bind to the same receptor on intestinal epithelial cells (Rabinowitz and Donnenberg, 1996).

The STI toxins produce a reversible short-term effect that is quick acting (within 5 minutes) and is mediated by guanylate cyclase. However, the biological effect of CT and LT is prolonged with a lag phase of about 1 hour, is reversible, and is mediated by activation of adenylate cyclase (Cohen and Gianella, 1995). LT-I and CT bind to adenylate cyclase from various tissues, whereas STI binds only to intestinal guanylate cyclase (Gyles, 1992).

STII appears to be primarily found in ETEC strains isolated from pigs. While STI is methanol soluble, STII is methanol insoluble. STII is a larger peptide than STI (5.1 kDa and 2 kDa, respectively), and does not cross-react immunologically with STI (O’Brien and Holmes, 1996). STII induces secretion of bicarbonate ions and water into the intestinal lumen, and increases the intracellular Ca^{2+} in intestinal cells (O’Brien and Holmes, 1996). STII does not appear to contribute to human disease (Salyers and Whitt, 1994); however, a few cases of STII-induced human diarrhea have been reported (Lortie et al., 1991; Okamoto et al., 1993). Reviews by Dubreuil (1997) and Nair and Takeda (1998) discuss various aspects of the heat-stable enterotoxins.

Other bacteria, including Citrobacter freundii, Yersinia enterocolitica and non-O1 Vibrio cholerae, produce toxins similar to STI (Smith, 1988; Chaudhuri et al., 1998). An STIa-containing plasmid from ETEC could be transferred to species of Shigella, Salmonella, Klebsiella, Enterobacter, Edwardsiella, Serratia and Proteus with stable maintenance of the plasmid and expression of toxin (Smith, 1988).
6.3.3 EAST1 (EAEC heat-stable toxin 1)

EAST1 is a heat-stable enterotoxin present in enteroaggregative *E. coli* (EAEC) strains (Savarino *et al.*, 1993). The gene for EAST1, *astA*, was found to be present in strains of ETEC isolated from both humans and animals (Yamamoto and Echeverría, 1996; Yamamoto and Nakazawa, 1997). The gene is present in piglet strains of ETEC producing STI, LT, or STI and LT, and was associated with adhesin factor K88. The K88 and EAST1 genes are on separate plasmids (Yamamoto and Nakazawa, 1997). In LT-, STI-, or LT- and STI-producing ETEC strains from humans, however, colonization factor antigens (CFA/I, CFA/II or CFA/IV) were associated with EAST1, and all genes were located on the same plasmid (Yamamoto and Echeverría, 1996).

Savarino *et al.* (1996) detected the EAST1 gene in 100% of 75 *E. coli* O157:H7 strains, in 47% of 227 EAEC, in 41% of 149 ETEC, in 22% of 65 EPEC strains, and in 13% of 70 DAEC strains, utilizing an *astA* DNA probe. In addition, *astA* was present in non-diarrhea-producing *E. coli* strains. Rich *et al.* (1999) found no correlation between the severity of diarrheic symptoms due to EAEC infection and the presence of EAST1. Thus, the *astA* gene appears to be common in *E. coli*; however, the significance of the EAST1 toxin in the pathogenesis of ETEC is unclear at the present time.

EAST1 is a low molecular weight, cysteine-rich, 38-amino-acid polypeptide enterotoxin that is plasmid encoded, partially heat stable (63% of activity remained after 65 °C for 15 minutes) and protease sensitive (Savarino *et al.*, 1991; O’Brien and Holmes, 1996; Ménard and Dubreuil, 2002). The EAST1 toxin does not cross-react serologically with STI; however, EAST1 shows homology with the receptor-binding domains of STI. Presumably the enterotoxin associates with the same receptor binding site of guanylate cyclase as STI, leading to cyclic GMP secretion (Savarino *et al.*, 1991; O’Brien and Holmes, 1996; Ménard and Dubreuil, 2002). Thus, EAST1 appears to be a member of the STI family of heat-stable enterotoxins and produces diarrhea by secreting cyclic GMP (Savarino *et al.*, 1993). A review by Ménard and Dubreuil (2002) summarizes various aspects of EAST1.

6.3.4 Colonization factors

The attachment of the ETEC strains to host cells is an important initial step in pathogenesis. The colonization factor antigens (CFAs), including CFAs I, II, and IV, are major adherence factors in human strains and are found only in diarrhea-causing ETEC (Salyers and Whitt, 1994). The CFA genes are plasmid encoded, and CFAs, ST and LT may be encoded by the same plasmid (Cohen and Giannella, 1995; Mol and Oudega, 1996). CFAs CFAII and CFAIV are further divided into CS (coli surface) antigens (Nirdnoy *et al.*, 1997). In addition to CFAI, CFAII, and CFAIV, a number of other CFAs have been described (Cassels and Wolf, 1995; Gaastra and Svennerholm, 1996; Grewal *et al.*, 1997; Ricci *et al.*, 1997). The CFA structures may be fimbrial rods, flexible fibrils, helical fibrils or curly fibrils (Cassels and Wolf, 1995). In 241 ETEC strains isolated from Mexican children, 46% of the strains possessed a CFA. Of LT/ST strains, 65% expressed a CFA, whereas 50% of ST and 25% of LT strains expressed a CFA (López-Vidal *et al.*, 1990). López-Vidal *et al.* (1990) found that children infected with ETEC lacking CFAs (I, II or IV) had diarrhea similar to those infected with CFA-containing ETEC. The fact that ETEC-lacking CFAs can
cause diarrhea suggests that unidentified colonization factors were responsible for
diarrhea in those ETEC strains.

Lyte et al. (1997) demonstrated that norepinephrine stimulated growth and
K99 pilus-mediated adhesion of ETEC. ETEC strains that express K99 pili are path-
genic for lambs, calves and pigs. The distal two-thirds region of the small intestine is
preferentially colonized by ETEC. This area is highly innervated with adrenergic
nerves, which produce norepinephrine at terminals present in the mucosal lining
(Lyte et al., 1997). While the K99 adhesion pili are virulence factors necessary for
colonization of ETEC in animals (Parry and Rooke, 1985), it is likely that intestinal
norepinephrine also stimulates induction of CFAs in human strains of ETEC.

6.4 ETEC and the immune response

Both purified CT and LT (or the B subunit of the toxins) have been used as oral
adjuvants, since they are potent mucosal immunogens. LT is less toxic than CT, and
can be used at levels that do not induce diarrhea (Baqar et al., 1995). CT induces a TH2
(T-helper cells involved in the humoral immune response) response with production of
IL-4 (interleukin 4) and IL-5 (interleukin 5) cytokines; in addition, the immunoglobu-
lins IgA, IgG1 and IgE are produced. LT, however, induces a mixed TH1 and TH2
response with production of IFN-γ (interferon-gamma), IL-4 and IL-5 cytokines.
LT induces an IgA, IgG1, IgG2 and IgG2b antibody response profile (Takahashi et al.,
1996). The IgE response induced by CT indicates that its use as a mucosal adjuvant
can lead to immediate-type allergic hypersensitivity, and the use of LT would be more desir-
able for this purpose. Oral administration of LT and heat-killed Campylobacter jejuni
stimulated both local and systemic Campylobacter-specific IgA and IgG in non-human
primates (Baqar et al., 1995). Co-administration of LT with oral inactivated influenza
vaccine to mice showed increased antiviral serum IgG and mucosal IgA as compared
to use of the vaccine alone (Katz et al., 1997). The use of LT as an oral adjuvant to
increase the secretion of secretory IgA on mucosal surfaces appears to be a viable
option in the control of human gastrointestinal and pulmonary diseases.

In developing countries, ETEC infections decrease as individuals become older –
which suggests the development of protective immunity against ETEC infections.
Therefore, it should be possible to develop vaccines against ETEC. Recent studies
have indicated that the oral administration of killed ETEC cells combined with
recombinant cholera-toxin B subunit to children or adults provided significant pro-
tection against ETEC infection (Savarino et al., 1999; Cohen et al., 2000; Qadri et al.,
2000). However, the development of an effective ETEC vaccine with broad protective
powers is difficult due to the large number of different intestinal adherence factors
expressed by ETEC strains (Nataro and Kaper, 1998).

Mason et al. (1998) constructed a synthetic gene coding for E. coli LT-B subunit
for use in transgenic potatoes. Feeding mice with raw tubers that expressed the LT-B
subunit protein resulted in high levels of serum and mucosal anti-LT-B immunoglob-
lulins. Plant-derived vaccine antigens, particularly produced in raw edible fruits,
should prove useful in protecting children and adults against diarrheic diseases
(Walmsley and Arntzen, 2000).
6.5 Detection of ETEC

There are no serological or biochemical markers to differentiate toxin-producing strains from non-toxigenic ETEC strains. Therefore, it is necessary to detect the toxins produced by ETEC strains. Nataro and Kaper (1998) described a number of molecular diagnostic techniques that can be used to detect LT and ST. A multiplex PCR allowing simultaneous detection of the ETEC LTI and STII genes was used to detect the pathogen in skim milk and porcine stool (Tsen et al., 1998). Monoclonal antibodies were produced against ETEC colonization factors and used to determine the prevalence of ETEC possessing the different colonization factors in children with diarrhea in Argentina (Viboud et al., 1993). López-Saucedo et al. (2003) described a single multiplex polymerase chain reaction that could be used to detect diarrheic E. coli, including enterotoxigenic E. coli. A DNA colony hybridization assay, including a pooled-toxin (STp, STh, and LT) probe assay and individual probe assays to detect toxins and a number of different colonization factors, was developed to detect and characterize ETEC (Steinsland et al., 2003).

7 Enteropathogenic *Escherichia coli* (EPEC)

7.1 Disease and epidemiology

Strains in the EPEC category are an important cause of infantile diarrhea in developing countries where water quality and hygiene are poor. The EPEC strains cause infections with high morbidity and mortality and are a threat to infants and young children worldwide. It is estimated that EPEC cause at least 117 million diarrheal episodes per year in developing countries (not including China) (Clarke et al., 2002). Outbreaks of diarrhea due to EPEC are rare in developed countries; however, outbreaks have occurred in day-care centers and pediatric wards (Vallance and Finlay, 2000). Transmission occurs primarily by the fecal–oral route, and contaminated hands, food and fomites serve as sources of infection. EPEC generally affects children less than 2 years of age, and especially infants less than 6 months of age. The diarrhea is self-limiting in most cases; however, in severe cases it can be prolonged, with wasting and failure to thrive (Fagundes-Neto and Scaletsky, 2000). Acute EPEC infections are manifested by profuse watery, mucoid (but non-bloody) diarrhea, often accompanied by vomiting and fever, and in severe cases death may result (Vallence and Finlay, 2000; Willshaw et al., 2000; Clarke et al., 2002). In adult volunteers, $10^8$–$10^{10}$ CFU are necessary to induce diarrhea; however, it is probable that the infectious dose in children is lower (Clarke et al., 2002). The incubation period for EPEC infection in children is unknown. Breastfeeding is protective, and infants generally become infected following weaning due to preparation of weaning foods with contaminated water. Oral hydration is the treatment of choice in mild cases, and parenteral rehydration is needed in severe cases. Children may suffer several diarrheal episodes each year due to EPEC, and no vaccines are currently available (Willshaw et al., 2000; Clarke et al., 2002). The reservoir for EPEC strains is the human gastrointestinal tract, and there is no evidence of zoonotic infections with human EPEC serotypes.
7.2 Basis of pathogenicity

The small-bowel epithelium is the site of EPEC infection (Vallance et al., 2002). EPEC strains bind loosely to the surface of small-bowel epithelial cells in a localized adherence pattern, and inject virulence factors into the cells. Disease is the result of the translocated bacterial virulence factors interacting with components of the host cells and altering the host-cell signaling pathways (Vallance and Finlay, 2000).

The EPEC adherence factor (EAF) plasmid is necessary for localized adherence. Densely packed three-dimensional clusters of bacteria adhering to the surface of tissue-culture cells is characteristic of localized adherence. The bundle-forming pilus (BFP), encoded by the EAF plasmid, is responsible for localized adherence, and is required for full virulence. BFP mutants are impaired in their ability to induce diarrhea (Frankel et al., 1998; Vallance and Finlay, 2000; Donnenberg and Whittam, 2001).

After initial attachment (localized adherence) of EPEC to the intestinal-cell membrane, proteins are secreted; this results in intimate bacterial attachment and the formation of cuplike pedestals on the microvilli on which the bacteria rest with the accumulation of polymerized filamentous actin, α-actinin, talin, ezrin and myosin light chain. These lesions are referred to as ‘attaching and effacing’ (A/E), and have been observed in vitro and in vivo (Donnenberg and Whittam, 2001). The A/E pathology is mediated by genes located on a 35-kb pathogenicity island, the locus of enterocyte effacement (LEE), which comprises 41 open reading frames. The G+C content of the LEE region is 38.4 %, in contrast to the E. coli chromosome, which has a G+C content of 50.8 % (Frankel et al., 1998). The LEE genes are separated into three domains: Tir (translocated intimin receptor) and the intimin outer membrane protein; EspA-D, encoding secreted proteins and their chaperones; and a region encoding a type-III secretion system, which translocates bacterial proteins directly into the host cell (Frankel et al., 1998; Donnenberg and Whittam, 2001). The Tir protein, encoded by LEE, is translocated via the type-III secretion system and is inserted into the host-cell plasma membrane. The EspS are involved in the translocation process. The inserted Tir is phosphorylated and then acts as the receptor for the intimin outer membrane protein. Intimin is the product of the eae gene located downstream of the tir gene in the LEE locus. Intimin is essential for intimate adherence and A/E formation (DeVinney et al., 1999a, 1999b; Donnenberg and Whittam, 2001). Thus, EPEC strains insert their own receptor (Tir) for the intimin adhesin protein, with resultant A/E lesion formation (DeVinney et al., 1999a).

A number of mechanisms have been proposed to explain how EPEC cause diarrhea; however, none of the proposed mechanisms have been studied in enough detail to elucidate the diarrheic mechanism (Nataro and Kaper, 1998; Vallance and Finlay, 2000). Thus, it is not clear how an EPEC infection actually triggers diarrhea.

Savarino et al. (1996) detected the EAST1 gene, astA, in 22 % of 65 EPEC strains; however, the significance of EAST1 toxin in these strains is unknown. Some EPEC strains that produce the A/E lesion have a gene (lifA) that encodes the toxin lymphostatin. Lymphostatin inhibits lymphocyte activation and selectively inhibits the production of IL-2, IL-4, IL-5 and IFN-γ. In addition, the toxin inhibits proliferation of lymphocytes (Klapproth et al., 2000). The expression of lymphostatin may
suppress the immune response against the bacteria and thereby prolong the infection, which would enhance the spread of the organism to other individuals.

### 7.3 Animal models

EPEC is a human pathogen, and does not infect most laboratory animals (Vallance and Finlay, 2000). However, A/E lesion-inducing *E. coli* strains have been isolated from rabbits (REPEC). The REPEC strains infect the small bowel of weanling rabbits and produce a disease similar to that caused by human EPEC. The rabbits suffer diarrhea and weight loss (DeVinney *et al.*, 1999a; Milon *et al.*, 1999). The pattern of adherence of REPEC is diffuse rather than localized as with human EPEC; however, the REPEC LEE-encoded secreted proteins are similar to those of human EPEC (Tauschek *et al.*, 2002). *Citrobacter rodentium* produces A/E lesions in mice but, unlike human EPEC and REPEC, *C. rodentium* colonizes the large bowel rather than the small bowel (Higgins *et al.*, 1999). The organism induces a TH1 response with production of interleukins, tumor necrosis factor alpha and gamma interferon. In addition, *C. rodentium* induces intestinal epithelial cell hyperplasia rather than diarrhea (Higgins *et al.*, 1999).

### 7.4 Detection of EPEC

Both A/E and the localized adherence phenotype of EPEC can be determined by the use of HEp-2 or HeLa cells (Nataro and Kaper, 1998). A fluorescence actin-staining assay has been used to detect A/E. This assay involves staining of actin that accumulates under the attached EPEC, using fluorescein-labeled phalloidin. Genotypic assays based on the use of DNA probes and the PCR have been described to evaluate the three major characteristics of EPEC: A/E (detection of the *eae* gene), presence of the EAF plasmid (EAF or *bfpA* gene probes), and the lack of Shiga toxin genes (use of gene probes or PCR primers targeting *stx*, discussed in Section 8 (Nataro and Kaper, 1998). López-Saucedo *et al.* (2003) described a multiplex PCR method for the detection of diarrheagenic strains of *E. coli*, including EPEC (gene targets for EPEC were *bfpA* and *eaeA*). A single multiplex PCR reaction could distinguish between EPEC, ETEC, EIEC and Shiga toxin-producing *E. coli* based on amplification of specific virulence genes.

### 8 Enterohemorrhagic *Escherichia coli* (EHEC)

Enterohemorrhagic *E. coli* (EHEC) were first identified as human pathogens in 1982, after the occurrence of outbreaks of hemorrhagic colitis due to consumption of undercooked hamburgers contaminated with *E. coli* O157:H7. The term ‘EHEC’ refers to *E. coli* serogroups including O26, O111, O103, O104, O118, O145 (with various H antigen types) and others that share the same clinical, pathogenic and epidemiologic features with *E. coli* O157:H7, the EHEC serotype that is responsible for the greatest proportion of disease cases. Since 1982 numerous outbreaks have been
documented, and it is estimated that \textit{E. coli} O157:H7 is responsible for greater than 73,000 cases of illness and 61 deaths each year in the United States (Mead \textit{et al}., 1999).

8.1 Disease characteristics

\textit{E. coli} O157:H7 can cause an asymptomatic infection; however, it usually leads to a mild non-bloody diarrhea or an acute grossly bloody diarrhea termed hemorrhagic colitis (HC) (Ryan \textit{et al}., 1986; Griffin, 1995; Su and Brandt, 1995; Mead and Griffin, 1998; Stephan \textit{et al}., 2000). The incubation period usually ranges from 3 to 8 days, but can be as short as 1–2 days. Illness in patients with non-bloody diarrhea is less severe, and these individuals are less likely to develop systemic sequelae or to die. HC is marked by an acute onset of severe abdominal cramps, followed by a progression of watery to bloody diarrhea that lasts for 4–10 days. The cecum and the ascending colon are the predominantly affected areas. Fever is usually absent or low-grade, stools are usually free of white blood cells, and about half of the patients have vomiting. With hemorrhagic colitis there may be elevation of blood leukocytes, edema with ‘thumb printing,’ hemorrhage of the lamina propria, superficial ulceration, pseudomembrane formation, and necrosis of the superficial colonic mucosa.

In 2–7\% of patients, predominantly infants and children, \textit{E. coli} O157:H7 infection can lead to hemolytic uremic syndrome (HUS) – a severe post-diarrheal systemic complication and the leading cause of acute renal failure in children in the United States (Mead and Griffin, 1998). The disorder is characterized by microangiopathic hemolytic anemia, thrombocytopenia and renal insufficiency (Su and Brandt, 1995). Central nervous system complications may occur in 30–50\% of patients. The production of Shiga toxins (Stx) by EHEC strains plays a large role in the pathogenesis of hemorrhagic colitis and HUS. Damage and death of endothelial cells through the action of Shiga toxins, which bind to specific receptors on endothelial cells, result in the deposition of platelets and fibrin, leading to abnormal white blood cell adhesion, reduced blood flow in small vessels of the affected organs, increased coagulation, and thrombus formation (O’Loughlin and Robins-Browne, 2001). As red blood cells and platelets pass through the narrowed blood vessels they are mechanically damaged, with resultant hemolytic anemia and thrombocytopenia. Histological changes in the kidney include capillary wall thickening, endothelial cell swelling, and thrombosis of capillaries in the glomeruli, resulting in necrosis of kidney tissue with complete occlusion of renal microvessels. Approximately 50\% of patients with HUS require dialysis; about 3–5\% die; and about 5\% develop chronic renal failure, stroke and other major sequelae (Mead and Griffin, 1998).

8.2 Basis of pathogenicity

Virulence factors of \textit{E. coli} O157:H7 and other typical EHEC strains include the production of one or more types of Shiga toxins, intestinal colonization and the production of A/E lesions as occurs with EPEC and mediated by genes located on the LEE locus, and the presence of a plasmid of approximately 60 MDa (pO157) (LeBlanc,
In the US, EHEC serotype O157:H7 is the most common cause of HC and HUS; however, numerous other serotypes produce Shiga toxins and have also caused HC and HUS. Thus, *E. coli* strains that produce Shiga toxins are referred to as Shiga toxin-producing *E. coli* (STEC). The most important virulence factors in the pathogenesis of EHEC infection are the Shiga toxins (Stx), which have also been referred to as verotoxins or verocytotoxins because of their cytopathogenic effect on Vero cells (African green monkey kidney cells). Cytotoxicity assays consisting of addition of serial dilutions of the samples to Vero cell monolayers, followed by examination of the cells by microscopy for cytotoxic effects, have been used for detection of the Shiga toxins. Shiga toxin is produced by *Shigella dysenteriae* type 1, and the gene is found on the chromosome. Shiga toxin 1 differs by only one amino acid from Shiga toxin, thus Stx1 was previously called Shiga-like toxin I. In *E. coli*, Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) are encoded on the genomes of temperate bacteriophages. Variants of Shiga toxin 1 and Shiga toxin 2 have been identified (Table 6.4; Schmidt *et al.*, 2000; Schmidt, 2001; Zhang *et al.*, 2002; Bürk *et al.*, 2003; Leung *et al.*, 2003). An STEC strain may produce Stx1, Stx2, or a combination of one or both toxins and one of the variants. Stx1c is associated with *E. coli* found in sheep, and the Stx2d variant was identified in an *E. coli* strain of bovine origin (Brett *et al.*, 2003; Bürk *et al.*, 2003). *E. coli* strains that produce Stx2e are responsible for edema disease in swine (Cornick *et al.*, 1999). The gene, stx2e, was believed to be chromosomally encoded; however, it was later found to be encoded on the genome of a Shiga toxin 2e-converting bacteriophage in an ONT (non-typable):H− *E. coli* strain isolated from a patient with diarrhea (Muniesa *et al.*, 2000).

Shiga toxins are composed of a single A polypeptide and a B-pentamer that binds to the eukaryotic cell receptor, globotriaosylceramide (Gb3), expressed on epithelial and endothelial cells; the receptor for Stx2e, on the other hand, is globotetraosylceramide (Gb4). After binding of the B-pentamer to the glycolipid receptors,

### Table 6.4  *E. coli* Shiga toxins

<table>
<thead>
<tr>
<th>Toxin type</th>
<th>Percent identity with relevant Stx</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><strong>A subunit</strong></td>
</tr>
<tr>
<td>Stx1a</td>
<td>–</td>
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<tr>
<td>Stx1c</td>
<td>97</td>
</tr>
<tr>
<td>Stx1d</td>
<td>93</td>
</tr>
<tr>
<td>Stx2</td>
<td>–</td>
</tr>
<tr>
<td>Stx2c</td>
<td>100</td>
</tr>
<tr>
<td>Stx2d</td>
<td>99</td>
</tr>
<tr>
<td>Stx2e</td>
<td>93</td>
</tr>
<tr>
<td>Stx2f</td>
<td>63</td>
</tr>
<tr>
<td>Stx2g (Vt2g)</td>
<td></td>
</tr>
</tbody>
</table>

a The A and B subunits of Stx1 are 55 % and 57 % identical to those of Stx2, respectively; Stx1 is 98 % identical to Stx of *Shigella dysenteriae*, differing by only one amino acid in the A subunit.

b The nucleic acid sequence of the vt2g A subunit showed 63 to 95 % similarity to other vt gene A subunit sequences; the nucleic acid sequence of vt2g B subunit showed 77 % to 91 % similarity to other vt gene B subunit sequences (Leung *et al.*, 2003).
the holotoxin is internalized by endocytosis via clathrin-coated pits. It is transported through the golgi network to the endoplasmic reticulum and the nuclear membrane. In the golgi, the 32-kDa A subunit is cleaved by a calcium-sensitive serine protease to an active 28-kDa peptide (A₁) and a 4-kDa peptide (A₂), but the fragments remain associated by a disulfide bond. In the endoplasmic reticulum, the disulfide bond linking the two peptides is reduced and the A₁ fragment is translocated into the cytoplasm. In the cytoplasm the A₁ subunit, an N-glycosidase, acts on the 60S ribosomal subunit, removing a single adenine residue from the 28S rRNA of eukaryotic ribosomes. Aminoacyl-tRNA then no longer binds to ribosomes, resulting in an irreversible inhibition of protein synthesis in eukaryotic cells. Recent studies have shown that Stxs can modulate the expression of chemokines and cytokines by human epithelial and endothelial cells, contributing to the inflammatory responses and the pathology of EHEC diseases (Cherla et al., 2003; Matussek et al., 2003). Furthermore, Stxs induce apoptosis (programmed cell death) in some cell types, which may contribute to the development of bloody diarrhea and HUS (Cherla et al., 2003).

Similar to the EPEC, *E. coli* O157:H7 and other EHEC possess the LEE locus and produce A/E lesions in the intestinal mucosa (LeBlanc, 2003). The *E. coli* O157:H7 LEE locus of *E. coli* O157:H7 strain EDL933 consists of 41 genes that are found in the same order and orientation as those in the EPEC O127:H6 strain. The average nucleotide identity between the LEE locus in the two strains is approximately 94%. The *esc* genes that encode the type-III secretion system are highly conserved; however, there is less similarity between the other genes including *eae*. The *eae* genes of EPEC and *E. coli* O157:H7 (strain 933) share 87% identity, with high conservation in the N-terminal region but variability in the C-terminal 280 amino acid region of intimin, which is involved in binding to enterocytes and Tir (Frankel et al., 1998). Both the EHEC and EPEC Tir bind intimin; however, the EHEC Tir is not tyrosine-phosphorylated, indicating that tyrosine phosphorylation is not required for A/E lesion formation (DeVinney et al., 1999c). At least 14 variants of intimin, including alpha 1, alpha 2, beta 1, beta 2, gamma 1, *eae*-xi and others have been identified using intimin type-specific PCR assays, and it has been suggested that different intimins in EPEC and EHEC may explain the different host tissue cell tropism (small bowel vs large bowel, respectively) (Frankel et al., 1998; Blanco et al., 2004).

Additional factors contributing to the virulence of EHEC are encoded on a ca. 60-MDa virulence plasmid. This plasmid is heterogenous and varies in size among EHEC strains, even within *E. coli* O157:H7 strains (LeBlanc, 2003). The complete DNA sequences of two pO157 virulence plasmids from strains EDL933 and RIMD 0509952 have been published (Burland et al., 1998; Makino et al., 1998). The plasmid from strain EDL 933 was a 92-kb F-like plasmid composed of 100 open reading frames, including the plasmid-encoded genes for the EHEC hemolysin (operon *ehxCABD*), which belong to the RTX family of exoproteins; KatP, a periplasmic catalase-peroxidase that functions to protect the bacterium against oxidative stress; a serine protease (EspP) that may contribute to the cytotoxic activity and tissue destruction by EHEC; and a gene cluster related to the type-II secretion pathway of Gram-negative bacteria (Etp system), composed of a cluster of 13 genes, *etpC*...
through etpO. Another unusually large ORF of 3169 amino acids showed strong sequence similarity within the first 700 amino acids to the N-terminal activity-containing domain of the large clostridial toxin (LCT) gene family in Clostridium difficile that includes ToxA and ToxB. Tatsuno et al. (2001) reported that toxB was required for adherence of E. coli O157:H7 to epithelial cells. One study showed that the EHEC enterohemolysin may contribute to the development of HUS through the production of IL-1β from human monocytes, and may mediate translocation of Stxs that stimulate the production of IL-1β (Taneike et al., 2002).

The low infectious dose of E. coli O157:H7 corresponds to the organism’s ability to tolerate acid environments, and the organism possesses at least three acid resistance systems (Lin et al., 1996; see ‘Growth and survival’, below). The TAT (twin arginine translocation) system may be another virulence factor of EHEC (Pradel et al., 2003). Deletion of the tatABC genes encoding the TAT system of E. coli O157:H7, involved in export of proteins across the cytoplasmic membrane, resulted in a decrease in secretion of Stx1 and abolished the synthesis of flagella. A cytolethal-distending toxin (cdt) gene cluster was identified in E. coli O157:H7 (6 % of isolates examined) and in sorbitol-fermenting E. coli O157:H- (87 % of isolates) isolated from patients with diarrhea and HUS (Janka et al., 2003). Their studies suggested that cdt may have been acquired by phage transduction.

E. coli O157:H7 releases membrane vesicles into the culture medium, which were shown to contain DNA encoding the eae, stx1, stx2 and uidA genes (Kolling and Matthews, 1999). Furthermore, E. coli O157:H7 vesicles facilitated the transfer of virulence and antibiotic resistance genes to other enteric bacteria, and the genes were expressed in the recipient bacteria (Yaron et al., 2000). Thus vesicle formation may be a mechanism for transport and transfer of genetic material and Shiga toxins. There is no evidence that E. coli O157:H7 is invasive in vivo; however, Matthews et al. (1997) showed that the pathogen invaded certain cell lines, including RPMI-4788 (human), MAC-T (bovine mammary secretory) and MDBK (bovine kidney, but not HeLa cells in vitro). The organism demonstrated both localized and diffuse adherence to the cells, and microtubules were required for invasion. The authors suggested that the ability to invade bovine mammary cells might be important in asymptomatic carriage of E. coli O157:H7 in cattle. Oelschlaeger et al. (1994) showed that E. coli O157:H7 invaded human ileocaecal (HCT-8) and bladder (T24) cell lines but not INT 407 intestinal cells. These investigators also reported that microfilaments were involved in internalization.

Quorum sensing is a phenomenon through which small signaling molecules, termed autoinducers, provide a means for cell–cell communication in response to cell population density. Quorum sensing may control virulence in E. coli O157:H7 by influencing transcription of genes in the LEE operon (Sperandio et al., 1999; Anand and Griffiths, 2003). Sperandio et al. (1999) suggested that intestinal colonization of EHEC might be regulated by quorum-sensing signals produced by non-pathogenic E. coli present in the intestinal flora. Using E. coli K12 DNA arrays, hybridization patterns of cDNA from RNA extracted from E. coli O157:H7 and from its isogenic luxS (gene involved in synthesis of autoinducer 2, AI-2) mutant showed up-regulation of 235 genes and down-regulation of 169 genes in the wild type strain compared to
the mutant (Sperandio et al., 2001). Up-regulated genes included those involved in chemotaxis, the SOS response and the synthesis of flagella and Stx. Thus, quorum sensing in *E. coli* O157:H7 is a global regulatory system. In a subsequent publication, Sperandio et al. (2003) reported that a molecule termed AI-3, and not the AI-2 molecule, is the signal involved in quorum sensing in EHEC. It was demonstrated that AI-3, whose synthesis also depends on LuxS, cross-talks with the mammalian hormone epinephrine, indicating that quorum sensing in *E. coli* O157:H7 may also involve bacterium–host communication.

### 8.3 Treatment and vaccines

Although there is no established therapy for *E. coli* O157:H7 infection, several vaccines are being developed and other promising regimens evaluated. The use of antibiotics in the treatment of infection is controversial, since antimicrobial therapy may increase the risk of development of HUS (Mølbak et al., 2002). Antibiotics may induce the expression of the Shiga toxins, and/or bacterial injury caused by the antibiotic may result in increased release of preformed toxins. Mulvey et al. (2002) suggested that administration of an Stx-binding agent, Synsorb-Pk, given in combination with antibiotics may potentially absorb sufficient amounts of toxin to prevent uptake into the circulatory system. This assumption requires testing in humans or in an appropriate animal model.

A number of vaccine protocols for use in cattle and humans are being investigated (Horne et al., 2002). A plant cell-based intimin vaccine tested in mice showed the development of an intimin-specific mucosal immune response and a reduced duration of shedding of *E. coli* O157:H7 (Judge et al., 2004). This plant-based vaccine system is being explored for oral administration to cattle to decrease shedding of the pathogen. Vaccination of cattle with type-III secreted proteins reduced the duration of shedding and numbers of *E. coli* O157:H7 in feces (Potter et al., 2004). Additionally, the prevalence of the organism in cattle was reduced in a clinical trial conducted under conditions of natural exposure in a feedlot setting. A vaccine consisting of liposomes incorporating monophosphoryl lipid A and antigens from an *E. coli* O157:H7 lysate induced IgG and IgA serum-antibody and mucosal-antibody responses in immunized mice (Tana et al., 2003). A number of other vaccine strategies, including toxoid and O-specific polysaccharide-protein conjugate vaccines, for prevention of EHEC disease are under investigation (Keusch et al., 1998; Konadu et al., 1998). Treatments based on use of Synsorb Pk, a synthetic analog of Shiga toxin receptor Gb3, bound to a calcinated diatomaceous material called Chromosorb are undergoing clinical trials (Takeda et al., 1999; Trachtman and Christen, 1999). Other proposed treatments include administration of recombinant bacteria expressing a Shiga toxin receptor mimic; humanized Shiga toxin-neutralizing monoclonal antibodies; pooled bovine colostrum containing antibodies to Shiga toxins, intimin, and the EHEC hemolysin; and bovine lactoferrin and its peptides; however, further studies are needed to elucidate the effects of these therapies in vivo (Shin et al., 1998; Huppertz et al., 1999; Paton et al., 2001; Yamagami et al., 2001).
8.4 Infectious dose

Analyses of foods implicated in disease outbreaks have revealed that the infectious dose for EHEC is less than 50 organisms (Tilden et al., 1996; Tuttle et al., 1999). The calculated number of *E. coli* O157:H7 found in raw ground beef patties implicated in an outbreak that occurred in the western US in November 1992 to February 1993 was 1.5 organisms per gram, or 67.5 per patty (Tuttle et al., 1999). In an outbreak that occurred in Australia associated with EHEC O111:H−, the contaminated, fermented sausages contained fewer than one *E. coli* O111:H− per 10 g (Paton et al., 1996). The occurrence of waterborne outbreaks, and outbreaks associated with visiting farms and petting zoos, in addition to person-to-person transmission of EHEC infection, provides further evidence of a low infective dose (Crump et al., 2002; O’Donnell et al., 2002; Olsen et al., 2002).

8.5 Antibiotic resistance

Over the past 20 years there has been an increase in antibiotic resistance observed in *E. coli* O157:H7 isolates (Schroeder et al., 2002a; Wilkerson and van Kirk, 2004). Resistance to tetracycline was the most common resistance found in bovine and human *E. coli* O157:H7 isolates, followed by resistance to streptomycin and ampicillin (Wilkerson and van Kirk, 2004). Mizan et al. (2002) found that antibiotic resistance plasmids could readily be transferred from a commensal *E. coli* strain to *E. coli* O157:H7 in bovine rumen fluid, at a frequency exceeding that observed for mating in LB broth. Antimicrobial resistance also appears to be widespread in non-O157 STEC (DeCastro et al., 2003; Schroeder et al., 2002b). Schroeder et al. (2002a) suggested that selection pressure imposed by the use of antimicrobials, including tetracycline derivatives, sulfa drugs, and penicillins in human and veterinary medicine, is resulting in the selection of antimicrobial resistant strains of STEC.

8.6 Animal models

A number of animal species have been evaluated as models of EHEC infection; however, in no animal system can the entire spectrum of the disease processes observed in humans be replicated. EHEC do not normally cause disease in cattle, but colostrum-deprived neonatal calves develop diarrhea 18 hours following inoculation with 10^{10} CFU of bacteria. The calves can become colonized with EHEC at levels greater than or equal to 10^6 CFU/g of intestinal tissue or feces, and may develop A/E lesions in the small or large intestine (Dean-Nystrom, 2003). Intimin was required for colonization of EHEC O157:H7 in newborn calves (Dean-Nystrom et al., 1998). Ritchie et al. (2003) used an infant rabbit model to study the role of *stx*₂, *eae* and *tir* in EHEC pathogenesis. EHEC derivatives with deletions in the *tir* and *eae* genes did not colonize or form A/E lesions, or cause inflammation and diarrhea. The *stx*₂ gene increased the severity and duration of diarrhea, but was not involved in attachment. Intragastric inoculation of Stx2 induced diarrhea and inflammation. Gnotobiotic
piglets infected orally with Stx2-producing *E. coli* O157:H7 and O26:H11 developed gastrointestinal illness and thrombotic microangiopathy in the kidneys, which is typically seen in humans with HUS (Gunzer *et al*., 2002). Comparing an *E. coli* O157:H7 strain with a mutation in the *eaeA* gene with the wild-type strain, Tzipori *et al.* (1995) showed that intimin (the product of *eaeA*) facilitated attachment to cells and affected the site of intestinal colonization. Pigs injected intramuscularly with Stx1 developed vascular damage and necrosis in the intestines and brain, similar to that which develops in humans with EHEC disease (Dykstra *et al*., 1993). The greyhound dog model is being investigated, due to similarities noted between EHEC disease and a condition in greyhounds called idiopathic cutaneous and renal glomerular vasculopathy (Fenwick and Cowan, 1998). Dogs with this illness exhibit renal changes similar to those seen in humans with HUS, and it is suspected that the disease in dogs is caused by Shiga toxin-producing *E. coli*. The disease was observed in dogs from which *E. coli* O157:H7 was isolated and in dogs administered Stx1 or Stx2 by intravascular inoculation. Baboons administered Stx1 by intravenous infusion develop renal failure and damage to the gastrointestinal mucosa (Melton-Celsa and O’Brien, 2003). The kidney lesions that develop are similar to those seen in kidneys of patients with HUS. A macaque monkey model is being developed in which the animals infected with *E. coli* O157:H7 acquire diarrhea and A/E lesions. Several mouse models have been developed to study EHEC pathogenesis (Melton-Celsa and O’Brien, 2003), and ferrets are being investigated as a model system to study EHEC-mediated HUS (Woods *et al*., 2002). Oral inoculation of chicks with *E. coli* O157:H7 showed that colonization occurred in the cecum and colon, and A/E lesions were detected in two out of seven chicks (Beery *et al*., 1985; Sueyoshi and Nakazawa, 1994). Use of appropriate animal models will advance our understanding of the pathophysiology of EHEC-induced HC and HUS, and provide information that will help to prevent, control and treat EHEC infection.

### 8.7 Growth and survival

Although there was some variation among strains, the minimum and maximum growth temperatures for *E. coli* O157:H7 studied in brain–heart infusion broth were 10˚C and 45˚C, respectively, and several strains grew slowly at 8˚C (Palumbo *et al*., 1995). Numerous studies have been conducted addressing the effects of environmental stresses and food production processes on the growth, survival and inactivation of *E. coli* O157:H7. *E. coli* O157:H7 has no unusual heat resistance; however, the thermal resistance can be influenced by a number of factors, including pH, growth conditions and growth phase of the cells, and the method of heating. Juneja *et al.* (1999) found that increasing the concentration of sodium pyrophosphate decreased the heat resistance; whereas increasing the concentration of NaCl increased resistance. The amount of fat in ground beef influences thermal tolerance of *E. coli* O157:H7. The *D* values for beef containing 2% fat and 3% fat were 4.1 and 5.3 minutes respectively at 57.2˚C, and 0.3 and 0.5 minutes respectively at 62.8˚C (Line *et al*., 1991).
López-González et al. (1999) showed that irradiation doses approved for red meats were sufficient to reduce the level of *E. coli* by several log values; however, the radiation temperature, oxygen permeability of the packaging material, and the medium in which the organism was irradiated influenced the $D_{10}$ values. Treatment consisting of dry heat in combination with an irradiation dose of 2.0 kGy was effective in eliminating *E. coli* O157:H7 from inoculated alfalfa and mung bean seeds, whereas a dose of 2.5 kGy was needed to eliminate the pathogen from radish seeds (Bari et al., 2003). Irradiation did not affect the germination percentage for alfalfa seeds or the length of the sprouts, but it did decrease the lengths of the radish and mung bean sprouts.

Although there is some variability among strains, *E. coli* O157:H7 is relatively acid-tolerant compared to other foodborne pathogens. The pathogen can grow at pH levels ranging from 4.4 to 9.0, and can survive for extended periods in foods at pH levels of 3.5–5.5. *E. coli* O157:H7 survived for up to 2 months, with only a 100-fold reduction in cell numbers during fermentation, drying and storage of fermented sausage; for 5–7 weeks in mayonnaise at 5°C; and for 10–31 days in apple cider at 8°C (Glass et al., 1992; Zhao et al., 1993, 1994). Application of warm (20°C) and hot (55°C) acetic, citric, and lactic acid sprays did not appreciably reduce the levels of *E. coli* O157:H7 on raw beef (Brackett et al., 1994). Three systems of acid tolerance have been characterized in *E. coli* O157:H7: an acid-induced, oxidative system requiring the Rpos alternate-sigma factor; an acid-induced, arginine-dependent system; and a glutamate-dependent system (Lin et al., 1996). Thus several acid resistance systems function in *E. coli* O157:H7, which permit survival under different acid-stress conditions, and once induced remain active during prolonged periods of cold storage.

*E. coli* O157:H7 survived in inoculated tap and bottled spring and mineral water for up to 300 days or more (Warburton et al., 1998), and for 14 days at <15°C in farm water stored outdoors, demonstrating the potential that farm water might serve as a vehicle for transfer of the organism in a herd (McGee et al., 2002). The organism survived for 77, >226 and 231 days in manure-amended autoclaved soil stored at 5°C, 15°C and 21°C, respectively (Jiang et al., 2002). It persisted for 25–41 days in fallow soils, 47–96 days on rye roots, and 92 days on alfalfa roots (Gagliardi and Karns, 2002). Persistence of the organism was not affected by the presence of manure, whereas the presence of clay increased persistence.

### 8.8 Sources of foodborne cases and outbreaks

Cattle are the major reservoir for *E. coli* O157:H7, and transmission of the pathogen from cattle to humans occurs via contaminated food or water. A survey conducted to determine the distribution and prevalence of *E. coli* O157:H7 in cattle in four major feeder-cattle states in the US showed that 10.2 % of fecal samples (out of 10 662 samples tested) and 13.1 % of water or water-tank sediment samples were positive, with over 60 % of feedlots having at least one positive water or water sediment sample (Sargeant et al., 2003). Elder et al. (2000) found an overall prevalence of *E. coli* O157:H7 or O157:NM, in the feces and hides of fed cattle presented for slaughter at meat processing plants in the midwestern US, of 28 % (91/327) and 11 % (38/355) in
feces and on hides, respectively – a prevalence much higher than previous studies had reported. The authors suggested that the isolation methods employed and time of year that the samples were collected were the likely reasons for the differences in results. Cattle harboring \textit{E. coli} O157:H7 are generally disease-free; however, the organism causes fatal ileocolitis in newborn calves. Tolerance to infection by \textit{E. coli} O157:H7 is likely due to lack of Gb$_3$, the Shiga toxin receptor, in the bovine gastrointestinal tract (Pruimboom-Brees \textit{et al.}, 2000). The duration of shedding of \textit{E. coli} O157:H7 in cattle is about 30 days (Sanderson \textit{et al.}, 1999), and the principal site of colonization is the terminal rectum (Grauke \textit{et al.}, 2002; Naylor \textit{et al.}, 2003). \textit{E. coli} O157:H7 has also been isolated from deer, pigs, horses, goats, sheep, cats, dogs, rabbits, poultry, and rats, and from birds such as ravens, doves, and seagulls (Meng \textit{et al.}, 2001; Feder \textit{et al.}, 2003). \textit{E. coli} O157:H7 strains administered to young adult sheep persisted longer in the gastrointestinal tract of the animals than did EPEC or ETEC strains that were included in the same inoculum (Cornick \textit{et al.}, 2000). The ability of EHEC to persist in ruminants may explain how this reservoir is maintained. The pathogen was isolated from houseflies collected from a school in Japan at which a disease outbreak occurred (Kobayashi \textit{et al.}, 1999). Feeding experiments showed that the bacteria were harbored in the fly’s intestine and were shed for at least 3 days after feeding, indicating proliferation of the organism in the fly.

Foods of bovine origin, including ground beef, raw milk and roast beef, have been associated with \textit{E. coli} O157:H7 infection; however, goat cheese, venison jerky, and environmental contamination with sheep feces have also been linked with outbreaks (Meng \textit{et al.}, 2001; Ogden \textit{et al.}, 2002). An outbreak affecting 732 individuals and with 55 cases of HUS and 4 deaths occurred in late 1992 and early 1993 in the western US and Canada (Bell \textit{et al.}, 1994). Inadequately cooked ground beef served at multiple outlets of the same fast-food restaurant chain was implicated as the cause of infection. Numerous other food vehicles, such as apple cider, mayonnaise, pea salad, cantaloupe, lettuce, hard salami, and alfalfa and radish sprouts, have also been linked to outbreaks (Meng \textit{et al.}, 2001). Fruits and vegetables are likely contaminated with cattle manure during harvesting and processing. Due to the ability of \textit{E. coli} O157:H7 to tolerate acidic environments, the organism can survive in foods of low pH such as apple cider or fermented products. White radish sprouts were implicated as the vehicle of infection in a large outbreak that occurred in Japan in 1996 that involved 9578 individuals, many of whom were schoolchildren, with 90 cases of HUS and 11 deaths (Bettelheim, 1997; Michino \textit{et al.}, 1999). Contaminated recreational water, well water, groundwater and municipal water systems have also been linked to outbreaks. In Walkerton, Ontario, Canada, in May 2000, an estimated 2300 individuals became seriously ill and 7 died due to exposure to drinking water contaminated with \textit{E. coli} O157:H7 from cattle excrement from a nearby farm that had washed into the town’s wells during a flood weeks earlier. Outbreaks resulting from visits to agricultural fairs and petting zoos have also occurred, likely due to exposure to animals – in particular cattle – and the farm environment (CDC, 2001; Crump \textit{et al.}, 2003). Additionally, person-to-person transmission of HC or HUS due to \textit{E. coli} O157:H7 infection occurs in nursing homes, day-care centers, and between family members (Al-Jader \textit{et al.}, 1999; Carter \textit{et al.}, 1987).
8.9 Diagnosis and methods for detection, isolation and identification of EHEC

Routine diagnosis of *E. coli* O157:H7 infection involves isolation of the pathogen, from stools of patients presenting with bloody diarrhea or HUS, on sorbitol MacConkey agar (SMAC). However, since *E. coli* O157:H7 also causes non-bloody diarrhea, it has been recommended that non-bloody stools from patients with diarrhea should also be cultured. Demonstration of the Shiga toxins in fecal filtrates is also useful for diagnosis; however, non-O157 STEC may also cause HC or HUS. Therefore, isolation and identification of the causative organism is necessary for epidemiological purposes. Different strategies for detection and isolation of *E. coli* O157:H7 in foods have been described (Fratamico *et al.*, 2002; Deisingh and Thompson, 2004). *E. coli* O157:H7 does not ferment sorbitol within 24 hours, thus colonies are colorless on SMAC. Sorbitol-negative colonies can be picked and characterized for responses to other biochemical parameters, for the presence of the O157 and H7 antigens, and for the presence of Shiga toxin genes or for the production of the Shiga toxins (Su and Brandt, 1995). Commercially available selective and differential agar media for isolation of *E. coli* O157:H7 include Rainbow® agar O157 (Biolog), CHROMagar® O157 (Hardy Diagnostics), BCM® O157:H7 Agar (Biosynth) and Fluorocult® *E. coli* O157:H7 Agar (Merck). Modifications of SMAC medium have resulted in agars with increased selectivity for *E. coli* O157:H7 or the ability to differentiate it from colonies of other organisms. These include CT-SMAC (which contains potassium tellurite and cefixime) or CR-SMAC (in which cefixime and rhamnose are added to SMAC), and SMA-BCIG containing the substrate for β-glucuronidase, 5-bromo-4-chloro-3-indoxyl-β-D-glucuronic acid cyclohexylammonium salt (BCIG). Presumptive positive colonies can be tested for the presence of the O157 and H7 antigens using commercially available latex agglutination kits or antisera, including the RIM® *E. coli* O157:H7 Latex Test (Remel) and the ImmunoCard STAT! *E. coli* O157 (Meridian Diagnostics) test.

Except for the production of Shiga toxins, there are generally no phenotypic markers (such as the inability to ferment sorbitol) that are shared by all non-O157 EHEC and that can be utilized as a strategy in the development of differential media to distinguish them from non-pathogenic *E. coli*. Thus, the incidence of disease caused by non-O157 EHEC may be underestimated due to the lack of adequate methods for detection of these pathogens. Methods for detection of non-O157 EHEC are described below.

Conventional methods rely on culture and agar media to grow, isolate and enumerate viable *E. coli* O157:H7. In recent years, however, numerous companies have developed methods for detection of *E. coli* O157:H7 and other pathogens that are specific, faster and often more sensitive than conventional methods. Immunoassays and genetic-based assays such as the polymerase chain reaction (PCR) are examples of rapid methods. Immunoassays rely on binding of an antibody to a specific bacterial antigen. A number of immunoassay formats, including enzyme-linked immunosorbent or fluorescent assays, lateral flow immunoassays and latex agglutination assays, for detection of *E. coli* O157:H7 are commercially available (Feng, 2001; Fratamico *et al.*, 2002).
Immunomagnetic separation (IMS) with magnetic particles coated with antibodies specific for *E. coli* O157 is available from Dynal, Inc., or the particles can be purchased from a number of vendors and the beads then coated with the desired antibody. Use of IMS on food enrichments and other complex matrices results in concentration of the target bacteria and sequestering from non-target organisms and other matrix components that interfere with subsequent detection systems (Fratamico and Crawford, 1999). IMS has been used in conjunction with plating onto selective agars and the PCR, and has been incorporated in commercially available kits including the PATH *IGEN™* *E. coli* O157 test (Igen International) and the EHEC-Tek™ for *E. coli* O157:H7 (Organon Teknika) for detection of the pathogen in foods (Fratamico and Crawford, 1999). Shiga toxins can be detected in bacterial culture supernatants, in food enrichments and in stool samples, using immunoassays — including colony immunoblot assays, latex agglutination, and antibody capture or toxin receptor-mediated enzyme linked immunosorbent assays. Immunoassay-based kits for the detection of Stx1 and Stx2 include the VTEC RPLA toxin detection kit (Oxoid), the Premier EHEC® (Meridian Diagnostics), and the RIDASCREEN® Verotoxin kit (r-biopharm). Nucleic acid-based detection systems, including PCR and DNA hybridization, rely on discrimination of *E. coli* O157:H7 from closely related organisms based on unique DNA or RNA sequences. Numerous PCR-based methods targeting genes including stx₁, stx₂, eae, uidA, hly, fliC, rfbE and others have been described (Deisingh and Thompson, 2004). PCR assays have also been performed in a multiplex format in which more than one sequence is amplified simultaneously in a single reaction (Fratamico et al., 2000; Campbell et al., 2001). Real-time PCR assays employing fluorogenic probes to visualize amplification of target sequences during the reaction have been applied for detection of *E. coli* O157:H7 (Ibekwe and Grieve, 2003; Sharma and Dean-Nystrom, 2003). Commercially available PCR-based assays include the BAX® *E. coli* O157:H7 (Dupont Qualicon), the Probelia PCR for *E. coli* O157:H7 (BioControl Systems), and the TaqMan® *E. coli* O157:H7 detection kit (Applied Biosoysystems). More recently, seven specific genes of *E. coli* O157:H7 were detected using oligonucleotide arrays (Liu et al., 2003). Biotin-labeled target DNA was obtained by incorporation of biotin-16-dUTP during multiplex PCR, and this was followed by hybridization to probes that were spotted onto glass slides and staining with streptavidin-Cy3. Call et al. (2001) used a combination of immunomagnetic capture and multiplex PCR, followed by detection of the PCR products using a microarray, to detect *E. coli* O157:H7 in rinse fluid from chickens at a level of 55 CFU/ml. Several other types of methods have also been described (Deisingh and Thompson, 2004).

### 8.10 Evolution of *Escherichia coli* O157:H7

The H7 flagellar gene (*fliC*) and *eae* gene sequences of *E. coli* O157:H7 and O55:H7 are nearly identical (Reid et al., 1999). Additionally, multilocus enzyme electrophoresis analyses have indicated that *E. coli* O157:H7 evolved from a progenitor strain with serotype O55:H7 (Feng et al., 1998). Through phylogenetic analyses based on enzyme allele profiles, a model for the stepwise emergence of *E. coli* O157:H7 was
formed. The immediate \textit{E. coli} O55:H7 ancestor that ferments sorbitol and expresses \( \beta \)-glucuronidase, evolved from an EPEC-like ancestor. The O55:H7 'clone' acquired the LEE pathogenicity island and a mutation at \(-10\) in the \( \beta \)-glucuronidase gene \textit{uidA}. Acquisition of the \textit{stx}\(_2\) gene by transduction by a toxin-converting bacteriophage resulted in the Stx2-producing \textit{E. coli} O55:H7 strain. A second mutation then occurred in the \textit{uidA} gene at +92, and the O antigen changed from O55 to O157, possibly by lateral transfer and recombination of a region of the \textit{rfb} locus containing the \textit{rfbE} gene that was homologous to the perosamine-synthetase gene of \textit{Vibrio cholerae}. The EHEC virulence plasmid was acquired at this stage. Two distinct lines then evolved from this progenitor: a non-motile sorbitol\(^+\) and \( \beta \)-glucuronidase\(^+\) Stx2-producing strain (O157:H\(^-\)), and a sorbitol-negative \( \beta \)-glucuronidase\(^+\) lineage that acquired the \textit{stx}\(_1\) gene (O157:H7, \textit{stx}\(_1^+\), \textit{stx}\(_2^+\)'). The latter lineage lost \( \beta \)-glucuronidase activity, producing the immediate ancestor of the O157:H7 'clone' that has spread worldwide. Comparison of \textit{gnd} gene sequences (located adjacent to the \textit{E. coli} O antigen gene cluster, also known as the \textit{rfb} locus) showed that \textit{gnd} co-transferred with the adjacent \textit{rfb} locus into \textit{E. coli} O157 and O55 in distantly separated lineages, and also that intragenic recombination may have contributed to allelic variation in this region of the O157 chromosome (Tarr \textit{et al}., 2000). L. Wang \textit{et al}. (2002) sequenced the \textit{E. coli} O55 O-antigen genes and flanking sequences to understand how the shift from O55 to O157 occurred. They identified two recombination sites, one within the \textit{galF} gene and the other between the \textit{hisG} and \textit{amn} genes, providing evidence for the recombination event proposed for the evolution of the \textit{E. coli} O157:H7 clone.

### 8.11 Genomic analysis of \textit{Escherichia coli} O157:H7

The genome of \textit{E. coli} O157:H7 EDL933 has been sequenced, providing information on the evolution of this organism (Perna \textit{et al}., 2001). In addition, the sequence data help in the identification of genes associated with virulence and in the development of methods for detection of the organism. Comparison of the O157:H7 genome to that of \textit{E. coli} K12 revealed that both share a similar backbone sequence of ca. 4.1 Mb; however, 1.34 Mb of DNA in \textit{E. coli} O157:H7 is missing in K12, and 0.53 Mb of DNA in K12 is missing in O157:H7 (Perna \textit{et al}., 2001; Sperandio, 2001). The \textit{E. coli} O157:H7 and K12 genomes consist of 5416 and 4405 genes respectively. Both O157:H7 and K12 are punctuated by hundreds of islands or DNA segments of up to 88 kb in length, designated K-islands in K12 and O-islands in O157:H7. Approximately 26 \% of the \textit{E. coli} O157:H7 genes are encoded within O-islands of diverse sizes, which are not found in K12. Only 40 \% of the O-island genes can be assigned a function. Putative virulence genes are encoded on nine large islands, while smaller islands encode genes involved in synthesis of fimbriae, in iron uptake and utilization, and in survival in different environments. Sequences related to known bacteriophages were identified in 18 multigenic regions. Together the sequence data indicate that there is a high level of diversity between the O157:H7 and K12 genomes, and that the islands were probably acquired through horizontal gene transfer from other organisms. Taylor \textit{et al}. (2002) showed that there was considerable variability in the presence, number and location of O-islands encoding tellurite resistance within \textit{E. coli} O157:H7 strains. Furthermore,
Shaikh and Tarr (2003) demonstrated that *E. coli* O157:H7 genomes possessed novel truncated bacteriophages and multiple *stx*₂ bacteriophage-insertion sites. Several antibiotics promoted excision of bacteriophages, and bile salts attenuated excision.

### 8.12 Genetic fingerprinting and outbreak investigation

Molecular typing methods are used to determine the genetic relatedness of bacterial isolates, to aid in epidemiologic investigations of foodborne disease outbreaks. Phenotypic methods such as serotyping, phage typing or multilocus enzyme electrophoresis are gradually being replaced by genetic fingerprinting techniques such as ribotyping, random amplified polymorphic DNA, and pulsed field gel electrophoresis (PFGE). The PFGE technique involves isolation of intact DNA, followed by digestion with restriction enzymes and analysis of the digestion products (typically 10–20 products ranging in size from 10 to 800 kb) that are separated by agarose gel electrophoresis with programmed variations in the direction and duration of the electric field. During an outbreak in 1993 caused by hamburgers contaminated with *E. coli* O157:H7 served at a fast-food restaurant chain, PFGE was used to determine the genetic relatedness of clinical and food isolates. All of the isolates associated with the multistate outbreak had identical phage type and PFGE patterns (Barrett *et al.*, 1994). It was determined that use of a standardized subtyping method would allow rapid comparison of isolates from different parts of the country and determination of a common source of infection, to prevent further spread of infection. Thus, a national molecular subtyping network for foodborne disease surveillance, known as PulseNet, was established by the Centers for Disease Control and Prevention in collaboration with the Association of Public Health Laboratories in 1996 (Swaminathan *et al.*, 2001). Laboratories participating in PulseNet perform PFGE on outbreak isolates from humans and/or the suspected food and enter the PFGE patterns into an electronic database to allow rapid comparison of the fingerprint patterns by the CDC. All 50 state public health laboratories, local public health laboratories, Food and Drug Administration and USDA Food Safety and Inspection Service laboratories participate in PulseNet, which is playing an integral role in the surveillance and investigation of outbreaks of foodborne illness caused by *E. coli* O157:H7 (CDC, 2002). PulseNet has been expanded and currently tracks non-typhoidal *Salmonella*, *Shigella*, *Listeria monocytogenes* and *Campylobacter*. Noller *et al.* (2003) reported that a subtyping technique known as multilocus variable-number tandem repeat analysis (MLVA), which targets short tandem repeats in the DNA at multiple loci, had a sensitivity equal to that of PFGE; however, specificity was superior to that of PFGE, since MLVA differentiated strains with identical PFGE types.

### 8.13 Importance of non-O157 STEC/EHEC

Over 200 STEC (also referred to as verocytotoxin-producing *E. coli* or VTEC) serotypes have been identified; 100 or more non-O157 O:H serotypes of STEC have been responsible for cases and outbreaks of HC and HUS (Nataro and Kaper, 1998; *World Health Organization*, 1998; Karmali, 2003; Table 6.5), and strains in this
subset of STEC are referred to as EHEC. The term ‘EHEC’ refers to STEC serotypes that share the same clinical, pathogenic and epidemiologic features with \textit{E. coli} O157:H7. Several important non-O157:H7 EHEC belong to \textit{E. coli} serogroups O26, O103, O111, O113, O121 and O145 (Nataro and Kaper, 1998).

In Australia, Latin America and many European countries, non-O157 STEC serotypes are more prevalent than \textit{E. coli} O157:H7 (Nataro and Kaper, 1998; Elliott \textit{et al.}, 2001; Werber \textit{et al.}, 2002; Karmali, 2003). Non-O157 STEC strains may account for 20–70\% of STEC infections throughout the world (World Health Organization, 1998). For example, \textit{E. coli} O121:H19 was linked to an outbreak of HUS at a lake in Connecticut (McCarthy \textit{et al.}, 2001); \textit{E. coli} O111:H\textsuperscript{−} was responsible for an outbreak in Australia involving 21 cases with 1 fatality, linked to consumption of a locally-produced semi-dry fermented sausage (Paton \textit{et al.}, 1996); and \textit{E. coli} O111:H8 caused a disease outbreak indistinguishable from that caused \textit{E. coli} O157:H7 in attendees at a youth camp (Brooks \textit{et al.}, 2004). \textit{E. coli} O26:H11 caused a multistate outbreak in Germany involving 11 case subjects and was linked to a certain type of beef referred to as \textit{Seemerrolle} (Werber \textit{et al.}, 2002).

Although non-O157 STEC have caused HC and HUS, infections with some STEC strains may result in asymptomatic or mild diarrhea. It is likely that these strains do not possess all of the virulence factors of \textit{E. coli} O157:H7, and further studies are needed to assess this possibility. In an outbreak linked to STEC O26:H11 in Germany, diarrhea was non-bloody and five persons remained asymptomatic (Werber \textit{et al.}, 2002). Friedrich \textit{et al.} (2002) found that STEC possessing different \textit{stx}\textsubscript{2} variants differed in their capacity to produce HUS. Strains harboring \textit{stx}\textsubscript{2c} caused HUS, whereas strains harboring \textit{stx}\textsubscript{2d} or \textit{stx}\textsubscript{2e} did not, although all strains caused

<table>
<thead>
<tr>
<th>Country</th>
<th>Serotype</th>
<th>Number affected</th>
<th>Vehicle of transmission</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>O103:H2</td>
<td>6</td>
<td>Not known</td>
<td>Mariani-Kurkdjian \textit{et al.}, 1993</td>
</tr>
<tr>
<td></td>
<td>O111:NM</td>
<td>9</td>
<td>Not known</td>
<td>Caprioli \textit{et al.}, 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Milk</td>
<td>CDC, 1995</td>
</tr>
<tr>
<td></td>
<td>O104:H21</td>
<td>11 (confirmed)</td>
<td>Uncooked, semi-dry fermented sausage</td>
<td>Paton \textit{et al.}, 1996</td>
</tr>
<tr>
<td>Australia</td>
<td>O111:H7</td>
<td>22</td>
<td>Not known</td>
<td>Hashimoto \textit{et al.}, 1999</td>
</tr>
<tr>
<td>Japan</td>
<td>O118:H2</td>
<td>126</td>
<td>Salad</td>
<td>Paton \textit{et al.}, 1999</td>
</tr>
<tr>
<td></td>
<td>O111:H8</td>
<td>58</td>
<td>Ice, salad bar</td>
<td>CDC, 2000</td>
</tr>
<tr>
<td>United States</td>
<td>O121:H19</td>
<td>11</td>
<td>Swimming water</td>
<td>McCarthy \textit{et al.}, 2001</td>
</tr>
<tr>
<td></td>
<td>O26:H11</td>
<td>4</td>
<td>Not known</td>
<td>McMaster \textit{et al.}, 2001</td>
</tr>
<tr>
<td>Ireland</td>
<td>O26:H11</td>
<td>6 (non-bloody diarrhea)</td>
<td>‘Seemerrolle’ beef</td>
<td>Werber \textit{et al.}, 2002</td>
</tr>
<tr>
<td>Germany</td>
<td>O26:H11</td>
<td>3</td>
<td>Not known</td>
<td>S (asymptomatic)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Misselwitz \textit{et al.}, 2003</td>
</tr>
</tbody>
</table>

Table 6.5  Outbreaks caused by non-O157 EHEC
diarrhea. Pradel et al. (2002) used a subtractive genomic hybridization technique to identify virulence genes specific to an *E. coli* O91:H21 strain that caused HUS. A comparison to the DNA of strains that were not associated with human illness showed that the O91:H21 strain possessed fragments corresponding to previously identified unique sequences in *E. coli* O157:H7, and sequences from *Shigella flexneri* and enteropathogenic and STEC plasmids; in addition, the strain possessed three copies of the *stx*2 gene. The authors suggested that highly pathogenic STEC strains acquire virulence genes by lateral gene transfer to a larger degree than do strains with lesser virulence.

An estimation of the true incidence of disease caused by the non-O157 STEC is complicated by the need to detect the presence of the Shiga toxins or of the *stx* genes. Unlike *E. coli* O157:H7, most non-O157 STEC cannot be detected based on selective and differential media that will detect the lack of the ability to ferment sorbitol or lack of β-glucuronidase activity. In addition to plating stool specimens from patients seen at the Inova Fairfax Hospital in Fairfax, Virginia, onto SMAC, Park and coworkers (2002) used a commercially available assay to detect the presence of Shiga toxins in the stool. *E. coli* O157:H7 was found in 45 out of 65 patient stool specimens, and non-O157 STEC strains were found in 20 specimens. The serotypes of the strains were O26:H12, O45:H2, O103:H2, O111:NM, O153:H2, O88:H25, O145:NM and O96:H9. Thus, they recommended that assays for the Shiga toxins be incorporated into clinical protocols for testing bloody stool specimens. This recommendation is also underscored by The Centers for Disease Control and Prevention.

A number of the O antigen gene clusters that contain genes involved in the synthesis of the O antigens of the different *E. coli* serogroups have recently been sequenced, and PCR assays targeting unique sequences within these regions have been used to detect specific *E. coli* serogroups (Fratamico et al., 2003; Wang et al., 2001b). For example, the *E. coli wzx* (O antigen flippase) and *wzy* (O antigen polymerase) genes are suitable targets for serogroup-specific PCR assays. Multiplex PCR assays targeting a serogroup specific region within the O antigen gene cluster, in addition to the *stx* or other virulence gene have also been reported (Wang, G. et al., 2002). A plating medium consisting of washed sheep’s blood agar and containing mitomycin C enhanced the ability to detect enterohemolysin-producing O157:H7 and non-O157 STEC strains (Sugiyama et al., 2001). Magnetic particles linked with antibodies specific for *E. coli* O26, O103, O111 and O145 are commercially available (Dynal). Other methods for detection of STEC, including immunological and DNA-based methods, have been described (Karch et al., 1999; Bettelheim, 2003).

Bibliography