Shiga Toxin–Producing *Escherichia coli*

Jenifer L. Jaeger, MD, and David W.K. Acheson, MD

Address
Division of Geographic Medicine and Infectious Diseases, New England Medical Center, 750 Washington Street, Boston, MA 02111, USA.
E-mail: david.acheson@es.nemc.org.

Current Infectious Disease Reports 2000, **2:**61–67
Current Science Inc. ISSN 1523–3847

Shiga toxin–producing *Escherichia coli* (STEC) are emerging as a significant source of foodborne infectious disease in the developed world. Multistate outbreaks of *E. coli* O157 and non-O157 serogroups in the United States are facilitated by the centralization of food processing and distribution. Our ability to recognize the clonality of these clusters has been advanced by developments in molecular detection techniques and in the establishment of active surveillance practices. These studies have helped identify important risk factors for both sporadic and outbreak STEC infection, allowing us to develop appropriate prevention strategies. Identification of these factors is of critical importance because of the lack of adequate treatments available. This brief review of the literature discusses major developments in the epidemiology, pathogenesis, diagnosis, treatment, and prevention of STEC disease published in the past few years.

Introduction

Shiga toxin–producing *Escherichia coli* (STEC), also known as verotoxin-producing *E. coli* and enterohemorrhagic *E. coli* (EHEC), are the cause of a significant emerging infectious disease. They were first described in 1983 after two outbreaks of hemorrhagic colitis in which a previously unrecognized pathogen, *E. coli* O157:H7, was linked to human illness. They were subsequently shown to make potent toxins called *Shiga toxins*, of which there are two main types: Shiga toxin 1 and Shiga toxin 2. In 1985, Karmali and coworkers showed an association between a variety of STEC serotypes, including O157:H7, and the hemolytic-uremic syndrome (HUS). STEC are a global problem, and more than 60 serotypes have been associated with human disease. O157:H7 is the serotype most often identified in humans in the United States, but other serotypes are more common in other countries.

This brief review summarizes important developments in the past 2 or 3 years that have advanced our understanding of STEC; the epidemiology and pathogenesis of STEC infection; and newer techniques in the diagnosis, treatment, and prevention of STEC disease. Several recent reviews offer the reader an overview of the clinical and microbiologic aspects of STEC and HUS. Two excellent reviews that discuss this subject in great detail are that by Paton and Paton [1••], which focuses on STEC infection specifically, and that by Nataro and Kaper [2••], which reviews the broader topic of diarrheagenic *E. coli*. These reviews elaborate on the role of plasmids in disease, the effect of toxins and other virulence factors, and the development of the intimate attachment lesions found in both EHEC and enteropathogenic *E. coli* (EPEC). In 1997, the Third International Symposium on Shiga Toxin–Producing *E. coli* was held in Baltimore, Maryland. Many of the presenters at that meeting have prepared papers on various aspects of STEC-related disease, and these papers have been compiled into an outstanding book edited by Kaper and O'Brien [3••]. This book is the most definitive source of current information on STEC.

Epidemiology

In the United States, implementation of the FoodNet surveillance system by the Centers for Disease Control and Prevention (CDC) has significantly enhanced our ability to determine the annual number of cases of infection by various foodborne pathogens, including *E. coli* O157:H7. Surveillance data from the CDC for the 1998 calendar year showed an increase in the number of confirmed outbreaks of *E. coli* O157:H7 infection, from an average of 31 per year between 1994 and 1997 to 42 in 1998. Outbreaks were seen in 25 states, for a total of 777 ill persons. Of these persons, 20% were hospitalized, 4% developed HUS, and 0.4% (three persons) died. Although contaminated ground beef still accounted for most outbreaks (this was confirmed in five outbreaks and suspected in six; one contact with cattle was noted; and five outbreaks were associated with dairy products), this report illustrates two recent developments in epidemiology. The first is the recognition of new and different vehicles for transmission: More and more outbreaks have been linked with foods other than ground beef and with water. The second is the widespread use of pulsed-field gel electrophoresis (PFGE).

In 1998, the CDC reported coleslaw to be the confirmed vehicle in two outbreaks and the suspected vehicle in one, and lettuce or salad was implicated in two outbreaks. Water was the source of infection in four outbreaks, including a large community outbreak in Wyoming due to contaminated drinking water and an outbreak from a water park in Georgia that led to one death. Person-to-per-

son contact was suspected or confirmed in seven outbreaks (day care centers were involved in six of the seven), and the source of transmission was unknown in 10 outbreaks. The importance of water as a source of *E. coli* O157:H7 infection is supported by a study showing that although this pathogen is not highly competitive in an aquatic environment, it can survive in a viable state for prolonged periods (weeks to months), even at cold temperatures [4]. Other reports of outbreaks of *E. coli* O157:H7 infection have implicated unpasteurized commercial apple juice and lettuce. Cattle are a well-recognized source of STEC, but other ruminants have been implicated. Sheep were found to be vehicles of pathogenic *E. coli*, with horizontal spread documented within flocks and long-term survival of *E. coli* O157:H7 noted in manure [5]. Interesting, but perhaps not surprising, is a report from Japan that showed transmission of *E. coli* O157:H7 by the common house fly [6].

Although in its infancy, the field of molecular epidemiology has greatly advanced our ability to identify outbreaks. Of particular interest is PFGE, which has facilitated the detection of epidemics by relating strains from different states and by demonstrating the clonality of small clusters of cases. The results of active PFGE surveillance by the Minnesota Department of Health in 1994 and 1995 were recently published [7•]. During the 2-year period, 344 cases were reported to the State Health Department and 317 were subtyped by PFGE; 143 distinct patterns were revealed. Ten outbreaks were recognized by this method; four were identified solely on the basis of PFGE. The clonal relationship among 54 EHEC isolates from Chile, serotypes O157, O111, and O26, was also characterized by PFGE [8]. Several clones were identified, including 12 distinct genetic profiles among the O157 isolates, indicating great diversity in the genotypes causing HUS. Analysis of virulence genes showed that 100% of the Chilean O157 isolates associated with HUS compared with 61.5% of isolates from asymptomatic carriers contained the *eae* locus.

Randomly amplified polymorphic DNA polymerase chain reaction (PCR) fingerprinting is another molecular tool that is being used more often [9]. By using specifically designed probes, both clonal relationships and the presence of virulence genes can be determined. Grif *et al.* [10] compared five methods for subtyping *E. coli* O157:H7 strains and concluded that PFGE, randomly amplified polymorphic DNA PCR, and phage typing were all valuable tools for epidemiologic surveillance. However, discrepancies among the various techniques suggest that isolates should not be classified by using a single method.

One facet of STEC that make them a particularly worrisome foodborne pathogen is their ability to survive the acidic milieu of various foods and the human stomach. This ability is attributed to the presumed acid-tolerance response, which allows the pathogens to resist extremely acidic conditions (pH, 2.0) when initially incubated at a more moderate pH. The ability to survive this food preservation technique has obvious implications. Brudzinski and Harrison [11] investigated the rate at which *E. coli* O157:H7 and *E. coli* non-O157:H7 become acid tolerant. They noted a wide range in tolerance among the various strains but showed (compared with controls) almost 1000-fold greater survival for O157:H7 isolates at a lower pH if the organisms were first allowed to gradually adapt to acid stress. These findings are supported by data in cattle showing a significantly lower pH concomitant with significantly more acid-resistant *E. coli*, including *E. coli* O157:H7, in the rumen and colonic digesta of animals fed a diet composed of a grain mixture (90% corn and soybean meal) compared with a diet composed entirely of hay [12•]. In contrast, a study found that sheep fed a diet of hay shed more *E. coli* and shed it for longer periods than did sheep fed a diet of corn and alfalfa [5]. The phenomenon of dry-resistant *E. coli* O157:H7—organisms able to survive on the surfaces of inert materials—was postulated on the basis of the high secondary transmission rate noted in a 1996 epidemic in Osaka, Japan [13]. Nonoutbreak strains showed markedly greater log reductions in growth secondary to dry stress compared with outbreak strains; one outbreak strain survived for 35 days under test conditions.

The low infective dose of *E. coli* O157:H7 (estimated to be as low as 10 cfu); the persistent virulence of these bacteria, even in the stressed state; and the ability of these bacteria to successfully adapt to stress underscore the importance of detection of these pathogens against a background of competing healthy bacteria. Selective culture on sorbitol-MacConkey agar (SMAC), which takes advantage of the serotype's inability to ferment sorbitol, does not support the growth of stressed organisms well. To address this problem, McCarthy *et al.* [14] developed an improved culture method designed to minimize the inhibitory effect of the selective agent in SMAC. Plating cells on a membrane-coated tryptone soy agar plate provides a resuscitation period. Subsequent transfer to SMAC compared with SMAC alone resulted in a marked increase in growth of acid-stressed *E. coli* O157:H7.

Much of our current understanding of the epidemiology of *E. coli* O157:H7 is derived from outbreak data. However, surveillance studies from the United States, Canada, and Europe reveal that most cases of *E. coli* O157:H7 infection are sporadic. Slutsker *et al.* [15•] published the first nationwide case–control investigation describing the epidemiology of *E. coli* O157:H7 infection. This prospective, multicenter study included 10 US hospitals during a 2-year period between 1990 and 1992. All submitted fecal specimens from inpatients and outpatients were cultured for *E. coli* O157:H7 and assessed for the presence of *stx*1 and *stx*2 genes. During the study period, *E. coli* O157:H7 was identified in 0.39% of the more than 30,000 specimens examined (118 persons); seven patients developed HUS, and one died.

On univariate analysis, significant risk factors included consumption of hamburger or hot dogs, eating in a fast food restaurant, drinking well water or swimming in a

pond, and having a household member with diarrhea. Only consumption of undercooked hamburger remained a significant risk factor on multivariate logistic analysis. Two features significantly associated with the development of HUS were identified: vomiting and use of antibiotics in children younger than 13 years of age within 3 days of onset of diarrhea. Of the six children who received antimicrobial agents within this period, five received an agent containing sulfamethoxazole and all five developed HUS. The effect of antimicrobial agents on the production of Shiga toxin and subsequent HUS is discussed in more detail below.

Surveillance data of outbreaks and sporadic cases of HUS have shown the increasing importance of non-O157:H7 serotypes worldwide. Current screening practices designed to identify O157:H7 do not select organisms that ferment sorbitol (*ie*, most non-O157:H7 serotypes), and the presence of other serotypes is often not sought, leading to underestimation of the potential effect of these serotypes. In France, six non-O157:H7 STEC strains producing Shiga toxin 2 were identified in 1996 and 1997 from sporadic cases of adult HUS [16]. The presence of the enterohemolysin-encoding gene varied, and the intimin-encoding gene was absent in all cases. A larger study of non-O157:H7 STEC from Germany in the same period confirmed the significance of the non-O157:H7 serotypes in human disease [17]. Almost 78% of 89 non-O157:H7 STEC isolates could be typed, revealing 15 different serotypes, and all produced Shiga toxin 1 or Shiga toxin 2. Most isolates produced enterohemolysins, but the presence of the intimin-encoding *eae* gene was a discriminating feature. The eae^+ genotype (seen in 61% of strains) was associated with more severe disease, including HUS, and young age. These studies illustrate two important aspects of STEC disease: the prominent new role of non-O157:H7 serotypes and the need to actively pursue the detection of these serotypes. The global extent of pathogenic non-O157 serotypes has been further shown by recent reports from France [18], Australia [19], New Zealand [20], and Germany [21]. It has been known for some time that STEC infection of the urinary tract may lead to HUS. Starr *et al.* [22•] wrote a review on this topic that illustrates the need to be cognizant of nonenteric sources of STEC in patients with HUS.

Pathogenesis

The mechanisms of STEC disease can be broadly divided into bacterial and host processes. Recent contributions to our understanding of the pathogenesis of STEC disease include studies on specific virulence factors, such as Shiga toxin and lipopolysaccharides, and on the ability to form attaching and effacing (A/E) lesions. The latter property requires host-bacterium interactions, including use of the host's signal transduction pathways.

Enterohemorrhagic and enteropathogenic *E. coli* share the histologic phenotype referred to as the *A/E lesion*. The ability to bind to the host intestinal epithelial cell membrane with subsequent effacement of microvilli and host cytoskeleton rearrangement permits the formation of microcolonies. Development of the A/E lesion requires numerous genes, including (but not only) *eae*, which encodes the bacterial outer membrane adhesion protein intimin; *tir*, which encodes the translocated intimin receptor; and a type III secretion system that secretes the *espA*-, *espB*-, and *espD*-encoded proteins EspA, EspB, and EspD. The A/E genes are located on a 35-kb chromosomal pathogenicity island termed the *locus on enterocyte effacement*. The importance of an intimate association between host and bacterium is supported by a study that characterized clinical isolates from a recent foodborne STEC outbreak. O serotypes associated with human disease, including O157, showed significantly greater adherence than did O serotypes isolated from food but not associated with infection [23].

Several studies have contributed substantially to our knowledge of the locus on enterocyte effacement. Abe *et al.* [24] created mutations in the *espA* and *espB* genes in an EPEC strain to evaluate A/E lesions in a rabbit model. Using histologic examination, scanning and transmission electron microscopy, and confocal laser scanning microscopy, these authors documented the extensive actin rearrangements in host epithelial cells beneath attached bacteria. Their results were supported by a study by Ebel *et al.* [25], who generated an EspA mutant for use in tissue culture with HeLa cells. They confirmed the role of EspA in inducing cytoskeletal changes and extended these findings by showing that EspA was probably involved in mediating initial binding to host epithelial cells. Kenny *et al.* [26•] showed that EPEC secrete a protein called translocated intimin receptor (Tir) that is inserted into the mammalian cell membrane and acts as the receptor for intimin. This property has also been found in certain STEC organisms, and amino acid sequence heterogeneity in both intimin and its receptor may contribute to the ability of STEC to avoid host defenses [27]. Dean-Nystrom *et al.* [28] investigated the role of *eae* in calves; those inoculated with an eae^- mutant *E. coli* O157:H7 strain showed no clinical or histopathologic abnormalities compared with those infected with an eae^+ *E. coli* O157:H7 strain. In the eae^+ animals, A/E lesions containing O157:H7 organisms were present in the ileum and colon. These data have important implications for vaccine strategies. Intimin vaccines may be useful in decreasing colonization and enterocolitis in calves and, thus, in reducing bacterial burden in humans. Unrelated to the locus on enterocyte effacement, but possibly contributing to the pathogenesis of STEC O157:H7 disease, is a secreted serine protease, EspP, that is encoded on the large plasmid present in many O157:H7 strains. This purported autotransporter cleaves pepsin and human coagulation factor V and therefore may affect the normal coagulation cascade, increasing gastrointestinal hemorrhage [29].

The role of bacteriophages in the pathogenesis of STEC disease has also been the focus of recent research. Lysogenic bacteriophages play a critical role in the dissemination of virulence genes to other *E. coli* as well as non-*Enterobacteriaceae* species. Acheson *et al.* [30] were able to demonstrate transduction of Shiga toxin 1–converting phage from one *E. coli* strain to another within the murine intestine. They had previously shown that induction of toxin-encoded bacteriophages generates an increase in the number of toxin gene copies. Production of infectious virions, therefore, is probably associated with a marked increase in toxin production. This was confirmed by Matsushiro *et al.* [31•], who found that norfloxacin induced Shiga toxin bacteriophages and elevated toxin production *in vitro*. Shiga toxin 2–converting bacteriophages have also been shown to exist as free particles in raw sewage, although the significance of this finding with regard to the transduction of other bacteria is uncertain [32]. Data continue to emerge on the sequencing of STEC. Recently, the sequences of the Shiga toxin 2 phage 933W [33] and the large virulence plasmid [34], both from *E. coli* O157:H7, were reported.

The role of the host immune system in the response to Shiga toxin has also been the focus of much research. Shiga toxins bind to specific glycolipid receptors; are internalized; and are translocated in a retrograde manner to the ribosome, where they inhibit protein synthesis. In addition to exerting this direct cytotoxic effect, they also seem to be able to stimulate pro- and anti-inflammatory cytokines and induce apoptosis. Shiga toxin cytotoxicity has been shown to be enhanced by tumor necrosis factor-α (TNF-α) in purified human glomerular microvascular endothelial cells [35] and human mesangial cells [36] from explanted kidneys. Isogai *et al.* [37] evaluated the role of TNF-α in EHEC infection in gnotobiotic mice. They detected TNF-α, interleukin-1α, and interleukin-6 in the kidney (and TNF-α in the brain) but not in serum after infection. Exogenous TNF-α was associated with worse clinical and histologic outcome, whereas a TNF-α inhibitor (a protease inhibitor) ameliorated these effects. These authors note that a TNF-α inhibitor combined with an inhibitor of Shiga toxin may be effective in reducing the serious sequelae of HUS.

Recent work to elucidate the molecular mechanisms of this relationship have determined that Shiga toxin 1 increases secretion of TNF-α through transcriptional activation. Sakiri *et al.* [38•] were able to show nuclear translocation of NF-κB and AP-1 in a human monocytic cell line, THP-1. This effect seems to be a specific response to Shiga toxin 1 because cyclohexamide, another protein synthesis inhibitor, had no effect on TNF-α production [39]. In contrast, protein kinase C, and not NF-κB, seems to be involved in the sensitization of human umbilical vein endothelial cells to Shiga toxin [40]. Several studies have measured cytokine levels in patients with *E. coli* O157:H7 HUS [41,42]. Although the varied clinical designs of these studies prohibit exact comparisons, the studies found elevated levels of circulating pro- and anti-inflammatory cytokines in persons with HUS compared with patients with less severe disease and healthy controls.

Thrombotic microangiopathy is pathognomonic of STEC-associated HUS and thrombotic thrombocytopenic purpura. Nitric oxide functions to mediate vascular tone and platelet aggregation and, therefore, its possible role in these disease processes has been explored. In a clinical study, Herlitz *et al.* [43] found indirect evidence for the activation of nitric oxide synthesis. In contrast, Bitzan *et al.* [44] showed an increase in preproendothelin-1 mRNA in response to Shiga toxin 1 or Shiga toxin 2 binding (an apparent effect of stabilizing labile mRNA) with no effect on nitric oxide synthase mRNA transcript levels. Additional research is needed to characterize these pathways more completely.

Diagnosis

With the emergence of *E. coli* O157:H7 as an important pathogen in sporadic and epidemic disease, much attention has been paid to the development of methods to better identify this organism. Screening cultures take advantage of the specific phenotypic characteristics of O157:H7, but these methods are slow and fail to identify non-O157:H7 serotypes (which are an increasing source of epidemics in many parts of the world) and thereby underestimate the effect of these serotypes on human disease. Polymerase chain reaction is a rapid, sensitive, and specific method that is being used more often to determine the presence of pathogens in humans, cattle, and food. In the past several years, numerous groups have designed multiplex PCR protocols that use two or more primer pairs to increase specificity for *E. coli* O157:H7 while maintaining the ability to detect other *E. coli* serotypes.

Gannon *et al.* [45] designed a multiplex PCR directed against EHEC and *E. coli* O157:H7 specifically. They designed two PCR primer pairs complementary to flanking regions of the *fliC* gene, which amplified fragments from all *E. coli* O157:H7 specimens tested but not from 49 *E. coli* strains of other H types. These oligonucleotide primers were used in conjunction with primers directed against *stx1*, *stx*2, and *eaeA*. Another method directed against *E. coli* O157:H7 uses PCR subtyping that relies on the amplification of variable sequences between repetitive sequences [46]. Insertion sequence 3 is typically found in multiple copies in most *E. coli* strains, and PCR conditions were optimized to allow discrimination of *E. coli* O157:H7 on the basis of DNA banding patterns. Although this PCR subtyping protocol was less sensitive than PFGE, its ease and the reproducibility of its results make it a useful screening tool for large numbers of samples.

Paton and Paton [47] developed two multiplex PCR assays to improve detection of STEC in feces and foods. The first uses four primer pairs for *stx1*, *stx2*, *eaeA*, and *hlyA* (a plasmid-encoded enterohemolysin); the second uses

two primer pairs specific for a portion of the *rfb* region (O antigen) of *E. coli* O157 and O111, another well-recognized cause of outbreak-associated bloody diarrhea and HUS. Modifications in primer sequences allowed detection of *stx2* variants, and the specificity of the O antigen primer set allowed for detection of all O157 and O111 strains tested without cross-reaction with the clonally related EPEC strain O55. Desmarchelier *et al.* [48] also focused on the O antigen of *E. coli* and designed a primer pair to amplify the *rfbE* O antigen synthesis gene. These primers cannot distinguish between strains that do and strains that do not produce Shiga toxin, but this could be modified by use in a multiplex system. Desmarchelier *et al.* further evaluated their primers in raw milk and found that the limit of detection was less than 1 cfu of *E. coli* O157:H7 per mL after enrichment.

Other diagnostic strategies have also been evaluated. The Premier EHEC assay (Meridian Diagnostics, Cincinnati, OH), an enzyme-linked immunosorbent assay for the detection of Shiga toxin 1 and Shiga toxin 2, was found to have a sensitivity of 100% and a specificity of 99.7%. Culture on SMAC, in comparison, has a sensitivity of 60% and a specificity of 100% [49]. It has a sensitivity similar to that of cytotoxicity assays but is considerably faster and easier to perform. Of note, 20% of childhood STEC infections in this study were caused by non-O157:H7 serotypes. The authors concluded that given the high risk for HUS in children, the Premier EHEC assay or another toxin-detection system should be considered part of standard enteric pathogen evaluation in this population. Other methods found to be rapid, sensitive, and specific— particularly at low levels of bacteria—include filtration capture combined with immunoelectrochemical detection [50]; Western blot assay for anti–Shiga toxin 1 IgG antibodies using chemiluminescence [51]; and Verotox-F (Denka Seiken, Tokyo, Japan), a system that uses anti–Shiga toxin 1 and 2 antibodies and compares favorably with Vero cell cytotoxicity assays [52]. Two techniques effective in detecting *E. coli* O157:H7 in foods, even at low inocula, are the BAX for Screening/*E. coli* O157:H7 (Qualicon, Wilmington, DE) [53] and a hydrophobic grid membrane filter, which was assessed in a multicenter study [54].

Treatment and Prevention

The standard of care for STEC infection in the United States does not include the use of antimicrobial agents. This practice is supported by in vitro studies showing an increase in Shiga toxin production from *E. coli* O157:H7 in the presence of subinhibitory concentrations of antibiotics. Shiga toxin genes are bacteriophage encoded; antibiotics that cause bacteriophage induction (*eg,* ciprofloxacin and trimethoprim-sulfamethoxazole) may increase Shiga toxin expression. Matsushiro *et al.* [31•] demonstrated induction of Shiga toxin phages and expression with norfloxacin. Fosfomycin, an inhibitor of peptidoglycan synthesis, is commonly used in Japan for diarrheal disease and STEC infection. Yoh *et al.* [55] noted an increase in Shiga toxin 1 release from *E. coli* O157:H7 in vitro in response to fosfomycin, but this toxin does not seem to cross an intestinal monolayer system [56]. A thorough review of the antibiotic susceptibilities of the O157:H7 isolates from the 1996 Japanese outbreak [57] revealed more than 90% inhibition of growth with fosfomycin at a dose of 0.5 μg/mL or less. Fosfomycin is not licensed in the United States for the treatment of diarrheal disease. Additional investigation is needed before current recommendations are changed.

Without a well-accepted treatment, prevention remains an important facet of STEC management. A substantial amount of research has been dedicated to identifying and eradicating STEC from beef during processing and in reducing STEC carriage in cattle. Duncan *et al.* [58] investigated the effect of dietary plant metabolites in the sheep rumen, pig gut, and human gut and found that coumarin esculin metabolized by colonic bacteria markedly reduced growth of *E. coli* O157:H7. Using an gnotobiotic mouse model, Isogai *et al.* [59] found that Japanese green tea extract inhibited the growth of *E. coli* O157:H7 in the gut and decreased the level of Shiga toxin in feces. A similar effect was seen with probiotic bacteria given to cattle [60].

Food safety initiatives are another area of investigation. Technologies currently available or on the horizon include electron-beam and γ-source irradiation and in-shell egg pasteurization [61]. A vaccine against the O-specific polysaccharide of lipopolysaccharide for O157 has successfully completed phase I trials in adults, and a phase II study in children is scheduled [62]. The final strategy for prevention rests in personal hygiene and measures for food preparation. A detailed discussion of suggestions for decreasing risk for foodborne illness, as well as information on the timing and clinical manifestations of foodborne diseases, can be found in *Safe Eating* [63]. Handwashing remains the most effective way to reduce person-to-person transmission of all enterically spread microorganisms. It is particularly important because the duration of fecal shedding of *E. coli* O157:H7 is longer than previously thought. A recent review of day care outbreaks shows prolonged shedding for as long as several weeks, particularly in younger children [64].

In summary, STEC disease, particularly *E. coli* O157:H7 infection, is an area of active investigation. Many laboratories have contributed significantly to our understanding of STEC, and this brief review of the recent literature presents only some of the latest developments. The greatest need at this point is to understand why only some exposed persons manifest disease and to develop adequate treatments to prevent the life-threatening complications of STEC infection.

References and Recommended Reading

Recently published papers of particular interest have been highlighted as:

- • Of importance
- •• Of major importance

1.•• Paton JC, Paton AW: **Pathogenesis and diagnosis of Shiga toxin-producing** *Escherichia coli* **infections.** *Clin Microbiol Rev* 1998, **11:**450–479.

This comprehensive review of Shiga toxin–producing *E. coli* infections focuses on the pathogenesis and diagnosis of these infections. Particular emphasis is placed on the role of Shiga toxins and other virulence factors of both O157 and non-O157 serotypes.

2.•• Nataro JP, Kaper JB: **Diarrheagenic** *Escherichia coli***.** *Clin Microbiol Rev* 1998, **11:**142–201.

This thorough analysis of diarrheagenic *E. coli* is organized according to the five major types of *E. coli*: enterotoxigenic, enteropathogenic, enterohemorrhagic, enteroaggregative, and enteroinvasive. In each section, the authors review the pathogenesis, epidemiology, and diagnosis unique and essential to the type being addressed. The paper also includes a separate discussion of diffusely adherent *E. coli*.

3.•• Kaper JB, O'Brien AD, eds.: *Escherichia coli O157:H7 and Other Shiga Toxin-Producing E. coli Strains.* Washington, DC: ASM Press; 1998.

This book contains the work presented at the Third International Symposium and Workshop on Shiga Toxin-Producing *Escherichia coli* Infections (VTEC '97), held in Baltimore, Maryland, 22–26 June 1997. It is the definitive source on *E. coli* O157:H7 and other STEC strains and reveals the diversity of disciplines involved in the study of STEC. The epidemiology of STEC in humans and in food, and the bacterial and host factors of pathogenesis, diagnosis, and treatment, including vaccine development, are all addressed in this single volume.

4. Wang G, Doyle MP: **Survival of enterohemorrhagic** *Escherichia coli* **O157:H7 in water.** *J Food Prot* 1998, **61:**662–667.

5. Kudva IT, Hunt CW, Williams CJ, *et al.*: **Evaluation of dietary influences on** *Escherichia coli* **O157:H7 shedding by sheep.** *Appl Environ Microbiol* 1997, **63:**3878–3886.

6. Iwasa M, Makino S, Asakura H, *et al.*: **Detection of** *Escherichia coli* **O157:H7 from Musca domestica (Diptera: Muscidae) at a cattle farm in Japan.** *J Med Entomol* 1999, **36:**108–112.

7.• Bender JB, Hedberg CW, Besser JM, *et al.*: **Surveillance for** *Escherichia coli* **O157:H7 infection in Minnesota by molecular subtyping.** *N Engl J Med* 1997, **337:**388–394.

This study illustrates the power of pulsed-field gel electrophoresis in relation to its ability to link apparently unconnected cases into outbreaks.

8. Rios M, Prado V, Trucksis M, *et al.*: **Clonal diversity of Chilean isolates of enterohemorrhagic** *Escherichia coli* **from patients with hemolytic-uremic syndrome, asymptomatic subjects, animal reservoirs, and food products.** *J Clin Microbiol* 1999, **37:**778–781.

9. Schmidt H, Geitz C, Tarr PI, *et al.*: **Non-O157:H7 pathogenic Shiga toxin-producing** *Escherichia coli***: phenotypic and genetic profiling of virulence traits and evidence for clonality.** *J Infect Dis* 1999, **179:**115–123.

10. Grif K, Karch H, Schneider C, *et al.*: **Comparative study of five different techniques for epidemiological typing of** *Escherichia coli* **O157.** *Diagn Microbiol Infect Dis* 1998, **32:**165–176.

11. Brudzinski L, Harrison MA: **Influence of incubation conditions on survival and acid tolerance response of** *Escherichia coli* **O157:H7 and non-O157:H7 isolates exposed to acetic acid.** *J Food Prot* 1998, **61:**542–546.

12.• Diez-Gonzalez F, Callaway TR, Kizoulis MG, *et al.*: **Grain feeding and the dissemination of acid-resistant** *Escherichia coli* **from cattle.** *Science* 1998; **281:**1666-1668.

This study investigates the phenomenon of acid-resistant *E. coli*. The authors raise the interesting possibility that feeding cattle a diet of hay before slaughter will diminish the virulence of *E. coli* O157:H7. This approach is fascinating, but more work is needed before it can be considered viable.

13. Iijima Y, Matsumoto M, Higuchi K, *et al.*: **Resistance to dryness of** *Escherichia coli* **O157:H7 strains from outbreak in Sakai City, Japan, 1996.** *Emerg Infect Dis* 1998, **4:**340–341.

14. McCarthy J, Holbrook R, Stephens PJ: **An improved direct plate method for the enumeration of stressed** *Escherichia coli* **O157:H7 from food.** *J Food Prot* 1998, **61:**1093–1097.

15.• Slutsker L, Ries AA, Maloney K, *et al.*: **A nationwide case-control study of** *Escherichia coli* **O157:H7 infection in the United States.** *J Infect Dis* 1998, **177:**962–966.

This collaborative study investigates the epidemiology of sporadic *E. coli* O157:H7 infection over a 2-year period across the United States. It enumerates risk factors for HUS, including the use of antimicrobial agents in children.

16. Bonnet R, Souweine B, Gauthier G, *et al.*: **Non-O157:H7 Stx2-producing** *Escherichia coli* **strains associated with sporadic cases of hemolytic-uremic syndrome in adults.** *J Clin Microbiol* 1998, **36:**1777–1780.

17. Beutin L, Zimmermann S, Gleier K: **Human infections with Shiga toxin-producing** *Escherichia coli* **other than serogroup O157 in Germany.** *Emerg Infect Dis* 1998, **4:**635–639.

18. Morabito S, Karch H, Mariani-Kurkdjian P, *et al.*: **Enteroaggregative, Shiga toxin-producing** *Escherichia coli* **O111:H2 associated with an outbreak of hemolytic-uremic syndrome.** *J Clin Microbiol* 1998, **36:**840–842.

19. Goldwater PN, Bettelheim KA: **New perspectives on the role of** *Escherichia coli* **O157:H7 and other enterohaemorrhagic E. coli serotypes in human disease.** *J Med Microbiol* 1998, **47:**1039–1045.

20. Brooks HJ, Bettelheim KA, Todd B, *et al.*: **Non-O157 Vero cytotoxin producing** *Escherichia coli***: aetiological agents of diarrhoea in children in Dunedin, New Zealand.** *Comp Immunol Microbiol Infect Dis* 1997, **20:**163–170.

21. Huppertz HI, Busch D, Schmidt H, *et al.*: **Diarrhea in young children associated with** *Escherichia coli* **non-O157 organisms that produce Shiga-like toxin.** *J Pediatr* 1996, **128:**341–346.

22.• Starr M, Bennett-Wood V, Bigham AK, *et al.*: **Hemolytic-uremic syndrome following urinary tract infection with enterohemorrhagic** *Escherichia coli***: case report and review.** *Clin Infect Dis* 1998, **27:**310–315.

This report of a case of HUS after urinary tract infection and review of 13 cases from the literature illustrates the danger of nonenteric infections with STEC leading to life-threatening complications.

23. Paton AW, Voss E, Manning PA, *et al.*: **Shiga toxin-producing** *Escherichia coli* **isolates from cases of human disease show enhanced adherence to intestinal epithelial (Henle 407) cells.** *Infect Immun* 1997, **65:**3799–3805.

24. Abe A, Heczko U, Hegele RG, *et al.*: **Two enteropathogenic** *Escherichia coli* **type III secreted proteins, EspA and EspB, are virulence factors.** *J Exp Med* 1998, **188:**1907–1916.

25. Ebel F, Podzadel T, Rohde M, *et al.*: **Initial binding of Shiga toxin-producing** *Escherichia coli* **to host cells and subsequent induction of actin rearrangements depend on filamentous EspA-containing surface appendages.** *Mol Microbiol* 1998, **30:**147–161.

26.• Kenny B, DeVinney R, Stein M, *et al.*: **Enteropathogenic** *E. coli* **(EPEC) transfers its receptor for intimate adherence into mammalian cells.** *Cell* 1997, **91:**511–520.

This study provides the first evidence of a bacterially encoded translocated protein in enteropathogenic *E. coli*. The authors determined the sequence of the intimin receptor and identified the presumed intimin binding site, two membrane-spanning domains, and the portion responsible for penetrating the host cell cytoplasm. This study has contributed greatly to our understanding of the pathogenesis of enteropathogenic *E. coli* and, subsequently, enterohemorrhagic *E. coli*.

27. Paton AW, Manning PA, Woodrow MC, *et al.*: **Translocated intimin receptors (Tir) of Shiga-toxigenic** *Escherichia coli* **isolates belonging to serogroups O26, O111 and O157 react with sera from patients with hemolytic-uremic syndrome and exhibit marked sequence heterogeneity.** *Infect Immun* 1998, **66:**5580–5586.

28. Dean-Nystrom EA, Bosworth BT, Moon HW, *et al.*: ***Escherichia coli* O157:H7 requires intimin for enteropathogenicity in calves.** *Infect Immun* 1998, **66:**4560–4563.
29. Brunder W, Schmidt H, Karch H: **EspP, a novel extracellular serine protease of enterohaemorrhagic** *Escherichia coli* **O157:H7 cleaves human coagulation factor V.** *Mol Microbiol* 1997, **24:**767–778.
30. Acheson DWK, Reidl J, Zhang X, *et al.*: **In vivo transduction with Shiga toxin 1-encoding phage.** *Infect Immun* 1998, **66:**4496–4498.
31.• Matsushiro A, Sato K, Miyamoto H, *et al.*: **Induction of prophages of enterohemorrhagic** *Escherichia coli* **O157:H7 with norfloxacin.** *J Bacteriol* 1999, **181:**2257–2260.

In a series of experiments, these authors documented the effects of sublethal concentrations of the new quinolone norfloxacin on toxin production from *E. coli* O157:H7. They showed induction of prophage with subsequent increase in toxin gene copy number and a significant increase in toxin. They further revealed involvement of the RecA system, helping to explain the effect of inhibition of DNA synthesis on toxin elaboration.

32. Muniesa M, Jofre J: **Abundance in sewage of bacteriophages that infect** *Escherichia coli* **O157:H7 and that carry the Shiga toxin 2 gene.** *Appl Environ Microbiol* 1998, **64:**2443–2448.
33. Plunkett G 3d, Rose DJ, Durfee TJ, *et al.*: **Sequence of Shiga toxin 2 phage 933W from** *Escherichia coli* **O157:H7: Shiga toxin as a phage late-gene product.** *J Bacteriol* 1999, **181:**1767–1778.
34. Burland V, Shao Y, Perna NT, *et al.*: **The complete DNA sequence and analysis of the large virulence plasmid of** *Escherichia coli* **O157:H7.** *Nucleic Acids Res* 1998, **26:**4196–4204.
35. van Setten PA, van Hinsbergh VWM, van der Velden TJAN, *et al.*: **Effects of TNFa on verocytotoxin cytotoxicity in purified human glomerular microvascular endothelial cells.** *Kidney Int* 1997, **51:**1245–1256.
36. Simon M, Cleary TG, Hernandez JD, *et al.*: **Shiga toxin 1 elicits diverse biologic responses in mesangial cells.** *Kidney Int* 1998, **54:**1117–1127.
37. Isogai E, Isogai H, Kimura K, *et al.*: **Role of tumor necrosis factor alpha in gnotobiotic mice infected with an** *Escherichia coli* **O157:H7 strain.** *Infect Immun* 1998, **66:**197–202.
38.• Sakiri R, Ramegowda B, Tesh VL: **Shiga toxin type 1 activates tumor necrosis factor-a gene transcription and nuclear translocation of the transcriptional activators nuclear factor-kB and activator protein-1.** *Blood* 1998, **92:**558–566.

This study establishes that Shiga toxins not only have direct cytotoxic effects but can also induce host cytokine defenses. In a series of well-controlled experiments, the authors show an increase in TNF-α mRNA mediated by the nuclear translocation of transcriptional activators. This further contributes to our understanding of the pathogenesis of STEC.

39. Hughes AK, Stricklett PK, Kohan DE: **Shiga toxin-1 regulation of cytokine production by human proximal tubule cells.** *Kidney Int* 1998, **54:**1093–1106.
40. Louise CB, Tran MC, Obrig TG: **Sensitization of human umbilical vein endothelial cells to Shiga toxin: involvement of protein kinase C and NF-kB.** *Infect Immun* 1997, **65:**3337–3344.
41. Proulx F, Turgeon JP, Litalien C, *et al.*: **Inflammatory mediators in** *Escherichia coli* **O157:H7 hemorrhagic colitis and hemolytic-uremic syndrome.** *Pediatr Infect Dis J* 1998, **17:**899–904.
42. van Setten PA, Hinsbergh VWM, van den Heuvel LPWJ, *et al.*: **Monocyte chemoattractant protein-1 and interleukin-8 levels in urine and serum of patients with hemolytic uremic syndrome.** *Pediatr Res* 1998, **43:**759–767.
43. Herlitz H, Petersson A, Sigström L, *et al.*: **The arginine-nitric oxide pathway in thrombotic microangiopathy.** *Scand J Urol Nephrol* 1997, **31:**477–479.
44. Bitzan MM, Wang Y, Lin J, *et al.*: **Verotoxin and ricin have novel effects on preproendothelin-1 expression but fail to modify nitric oxide synthase (ecNOS) expression and NO production in vascular endothelium.** *J Clin Invest* 1998, **101:**372–382.
45. Gannon VPJ, D'Souza S, Graham T, *et al.*: **Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic** *Escherichia coli* **strains.** *J Clin Microbiol* 1997, **35:**656–662.
46. Thompson CJ, Daly C, Barrett TJ, *et al.*: **Insertion element IS3-based PCR method for subtyping** *Escherichia coli* **O157:H7.** *J Clin Microbiol* 1998, **36:**1180–1184.
47. Paton AW, Paton JC: **Detection and characterization of Shiga toxigenic** *Escherichia coli* **by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic** *E. coli* **hlyA, rfbO111 and rfbO157.** *J Clin Microbiol* 1998, **36:**598–602.
48. Desmarchelier PM, Bilge SS, Fegan N, *et al.*: **A PCR specific for** *Escherichia coli* **O157 based on the rfb locus encoding O157 lipopolysaccharide.** *J Clin Microbiol* 1998, **36:**1801–1804.
49. Kehl KS, Havens P, Behnke CE, *et al.*: **Evaluation of the Premier EHEC assay for detection of Shiga toxin-producing** *Escherichia coli***.** *J Clin Microbiol* 1997, **35:**2051–2054.
50. Brewster JD, Mazenko RS: **Filtration capture and immunoelectrochemical detection for rapid assay of** *Escherichia coli* **O157:H7.** *J Immunol Methods* 1998, **211:**1–8.
51. Reymond D, Karmali MA, Clarke I, *et al.*: **Comparison of the Western blot assay with the neutralizing-antibody and enzyme-linked immunosorbent assays for measuring antibody to Verocytoxin 1.** *J Clin Microbiol* 1997, **35:**609–613.
52. Karmali MA, Petric M, Bielaszewska M: **Evaluation of a microplate latex agglutination method (Verotox-F assay) for detecting and characterizing Verotoxins (Shiga toxins) in** *Escherichia coli***.** *J Clin Microbiol* 1999, **37:**396–399.
53. Johnson JL, Brooke CL, Fritschel SJ: **Comparison of the BAX for screening/E. coli O157:H7 method with conventional methods for detection of extremely low levels of** *Escherichia coli* **O157:H7 in ground beef.** *Appl Environ Microbiol* 1998, **64:**4390–4395.
54. Entis P: **Direct 24-hour presumptive enumeration of** *Escherichia coli* **O157:H7 in foods using hydrophobic grid membrane filter followed by serological confirmation: collaborative study.** *J AOAC Int* 1998, **81:**403–418.
55. Yoh M, Frimpong EK, Honda T: **Effect of antimicrobial agents, especially fosfomycin, on the production and release of Vero toxin by enterohaemorrhagic** *Escherichia coli* **O157:H7.** *FEMS Immunol Med Microbiol* 1997, **19:**57–64.
56. Izumikawa K, Hirakata Y, Yamaguchi T, *et al.*: *Escherichia coli* **O157 interactions with human intestinal Caco-2 cells and the influence of fosfomycin.** *J Antimicrob Chemother* 1998, **42:**341–347.
57. Tsuboi I, Ida H, Yoshikawa E, *et al.*: **Antibiotic susceptibility of enterohemorrhagic** *Escherichia coli* **O157:H7 isolated from an outbreak in Japan in 1996.** *Antimicrob Agents Chemother* 1998, **42:**431–432.
58. Duncan SH, Flint HJ, Stewart CS: **Inhibitory activity of gut bacteria against** *Escherichia coli* **O157 mediated by dietary plant metabolites.** *FEMS Microbiol Lett* 1998, **164:**283–288.
59. Isogai E, Isogai H, Takeshi K, *et al.*: **Protective effect of Japanese green tea extract on gnotobiotic mice infected with an** *Escherichia coli* **O157:H7 strain.** *Microbiol Immunol* 1998, **42:**125–128.
60. Zhao T, Doyle MP, Harmon BG, *et al.*: **Reduction of carriage of enterohemorrhagic** *Escherichia coli* **O157:H7 in cattle by inoculation with probiotic bacteria.** *J Clin Microbiol* 1998, **36:**641–647.
61. Tauxe RV: **Strategies for surveillance and prevention.** *Lancet* 1998, **352(suppl IV):**10.
62. Konadu EY, Parke JC Jr, Tran HT, *et al.*: **Investigational vaccine for** *Escherichia coli* **O157: phase 1 study of O157 O-specific polysaccharide-***Pseudomonas aeruginosa* **recombinant exoprotein A conjugates in adults.** *J Infect Dis* 1998, **177:**383–387.
63. Acheson DWK, Levinson RK: *Safe Eating.* New York: Dell Publishing; 1998.
64. Swerdlow DL, Griffin PM: **Duration of faecal shedding of** *Escherichia coli* **O157:H7 among children in day-care centres.** *Lancet* 1997, **349:**745–746.