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Shiga Toxin–Producing *Escherichia coli* (STEC)

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KEYWORDS

• Escherichia coli • STEC • Enterohemorrhagic • Verotoxigenic
• Diarrhea • Hemolytic uremic syndrome

Shiga toxin–producing *Escherichia coli* (STEC) comprise one of four generally recognized groups of *E coli* causing diarrhea in humans who acquire infections by ingestion of contaminated food or water or another fecal-oral route. The natural reservoir for STEC is ruminant animals, notably cattle, in which STEC can occur as normal intestinal flora (**Fig. 1**).[1,2] STEC is unique among these *E coli* by virtue of harboring and expressing the genes for Shiga toxins type 1 (Stx1) and 2 (Stx2). Shiga toxin is named for the Japanese microbiologist Kiyoshi Shiga (1870–1957), for whom the genus *Shigella* is named, inasmuch as the toxin produced by *Shigella dysenteriae* type 1 is very similar to the Stx1 and Stx2 produced by STEC. **Table 1** provides a brief summary of these four well-characterized diarrheogenic *E coli*. They are distinct from the commensal *E coli* strains that inhabit the human gut and cause urinary tract infections, bacteremia, meningitis, and pneumonia in susceptible patients.[3]

Transmission of STEC to humans occurs through consumption of undercooked ground (minced) beef, foods eaten raw (eg, lettuce, sprouts, or spinach from manured gardens), water, or unpasteurized milk or juices, contaminated with STEC originating from cattle feces.[4] Direct animal-to-human and human-to-human transmission have occurred.[4,5] Worldwide, STEC are often referred to as ''verotoxin-producing'' or ''verocytotoxin-producing'' *E coli* (VTEC), making reference to their cytotoxic effect on the Vero monkey kidney cell line (**Fig. 2**).[6] In older literature, STEC was referred to as ''enterohemorrhagic *E coli*'' (EHEC), referring to bloody stools that are often a part of the clinical presentation,[4,7] and use of the EHEC acronym continues.

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Fig. 1. Cattle and other ruminants are natural reservoirs of STEC. (*Courtesy of* Sarah Hunt, St Paul, MN.)

HISTORICAL EMERGENCE OF STEC AS A DIARRHEAL PATHOGEN

Reported as *E coli* strains having distinct O-antigenic serotypes associated with bloody diarrhea and postdiarrheal hemorrhagic colitis in children in Canada[6] and in the United States,[8] STEC has emerged as a frequent cause of food-borne gastroenteritis, both sporadically and in outbreaks, creating substantial risk of hemolytic uremic syndrome (HUS) and life-threatening renal failure in children.[9] Over the past 25 years, most attention has been focused on one particular STEC O-antigen serotype, O157, of flagellar serotype H7 (*E coli* O157:H7, or STEC O157), which predominates in reported outbreaks and sporadic cases in the United States and elsewhere worldwide, although not universally.[4] In Germany, for example, STEC O91 is the predominant serotype isolated from adult patients.[10] STEC O157 is notable for having caused an outbreak of diarrhea in the United States in 1982 traced to contaminated beef served by a fast-food restaurant chain that resulted in four deaths[8]; and a 1996 Japanese outbreak affecting an estimated 6000 children, three of whom died.[11]

The two toxins that can be produced by STEC have been referred to historically as ''Shiga-like toxins,'' and ''verotoxins'' or ''verocytotoxins.'' The acronyms STEC, EHEC, and VTEC are used interchangeably. Stx1 differs in one amino acid from Shiga toxin of *Shigella dysenteriae* serotype 1, whereas Stx2 shares only about 60% amino acid similarity with Stx1. Sequence variants of both Stx1 and Stx2 are known, and multiple variants may be produced by one STEC bacterium.[12]

With heightened awareness of the importance of STEC O157, and improvements in its laboratory detection in stool specimens,[3] sporadic infections and outbreaks of varying magnitude continue to be documented. Laboratory testing has improved to the point that a standard of care is to always suspect and test for this particular pathogen in stool specimens from patients with diarrhea. Importantly, it is the clinician's responsibility to communicate with the clinical microbiology laboratory to ensure that appropriate specimen collection and transport occurs, and that cultures are ordered and performed to recover and isolate STEC O157 from stool specimens submitted for bacterial cultures.[3,13] Isolation of a pure culture of STEC from patients

Table 1
Four major groups of diarrheogenic *E coli*

Diarrheogenic *E coli*	Toxins	Other Virulence Factors	Worldwide Disease Burden	Clinical and Public Health Aspects
STEC (EHEC, VTEC)	Produces Shiga toxins Stx1, Stx2 (Verotoxins)	Survival in undercooked beef, on raw vegetables, in milk, and in water and fruit juices; adhesins for adherence to intestinal epithelium	Significant cause of HUS following bloody or nonbloody diarrhea; O157:H7 most prevalent, but STEC non-O157 important	A zoonotic disease acquired from foods and water contaminated with feces of cattle and other ruminants; secondary cases likely; low infectious dose
EIEC	None	Low infectious dose; invades intestinal epithelium	Rare; endemic in some countries; localized outbreaks in nurseries	Similar to Shigella, with fever, pain, dysentery; food-borne and person-to-person transmission
EPEC	None	Protein factors for attachment to and effacement of enterocyte microvilli; distinct pili for attachment to enterocytes	Significant cause of infant (<1 y) diarrhea; associated with weaning in infants; dehydration may be severe and fatal	Watery diarrhea with mucus, fever, with nausea and vomiting; foodborne transmission
ETEC	Heat-labile toxin (LT), heat-stable toxin (ST)	Colonization factors (proteins) expressed in intestinal lumen	Many fatal cases in children <5 y; associated with weaning in infants; dehydration may be severe	Profuse watery diarrhea; usually self-limiting as a common complaint of adult travelers

Abbreviations: STEC (EHEC, VTEC), Shiga toxin–producing *E coli* (enterohemorrhagic *E coli*, verocytotoxin- or verotoxin-producing *E coli*); EIEC, enteroinvasive *E coli*; EPEC, enteropathogenic *E coli*; ETEC, enterotoxigenic *E coli*; HUS, hemolytic-uremic syndrome.

Data from Nataro JP, Bopp CS, Fields PI, et al. *Escherichia*, *Shigella*, and *Salmonella*. In: Murray PR, Baron EJ, Jorgensen JH, et al, editors. Manual of clinical microbiology. 9th edition. Washington, DC: American Society for Microbiology; 2007. p. 670–87; Fontaine O, Griffin P, Henao O, et al. Diarrhea, acute. In: Heymann DL, editor. Control of communicable diseases manual. 19th edition. Washington, DC: American Public Health Association; 2008. p. 179–95; Centers for Disease Control and Prevention. Diagnosis and management of foodborne illnesses: a primer for physicians and other health care professionals. MMWR Morb Mortal Wkly Res 2004;53:1–33.

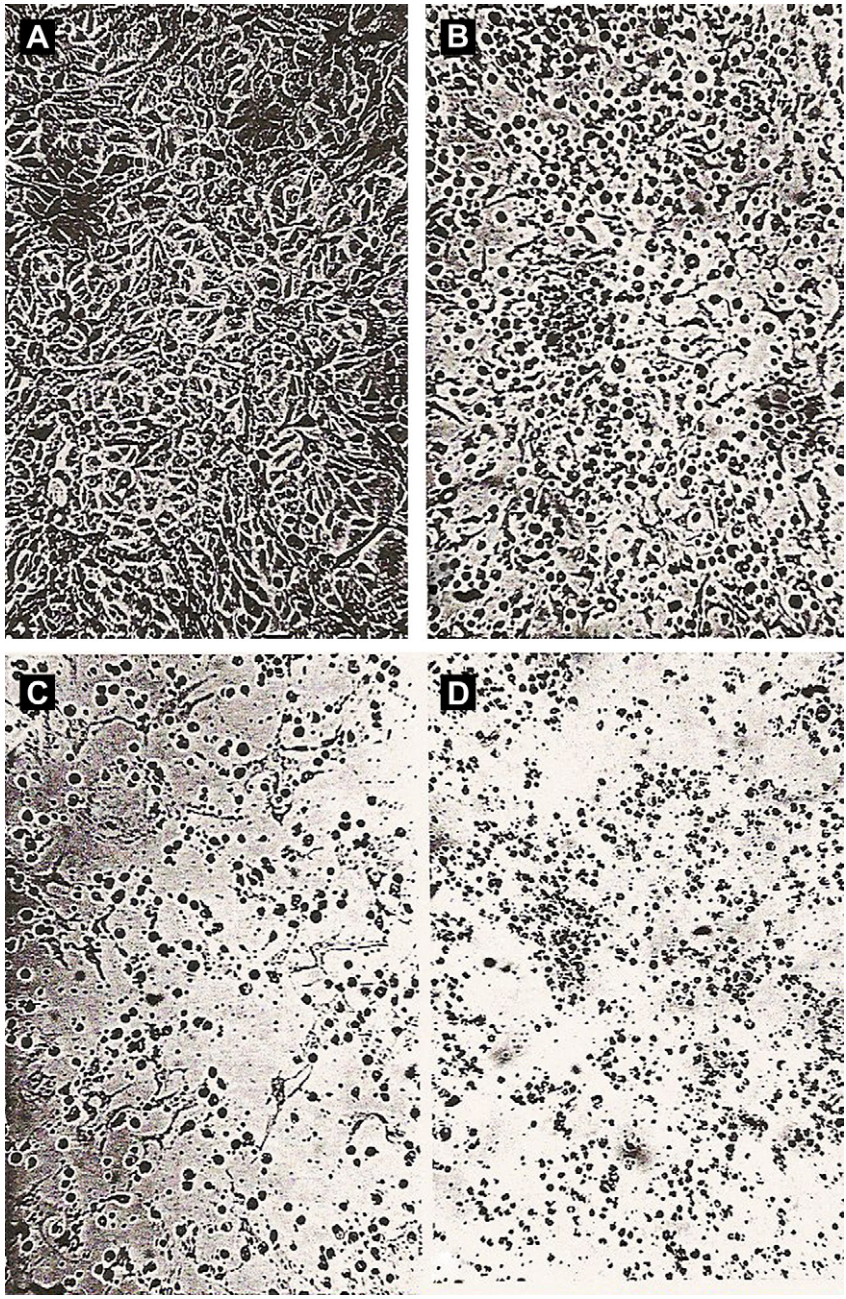

Fig. 2. Shiga toxin (Verotoxin) effect on Vero cell monolayers. (*A*) Medium control, 1 day, cells in continuous monolayer. With STEC filtrate, (*B*) 1 day, (*C*) 2 days, (*D*) 5 days; monolayer being destroyed. Phase-contrast microscopy. Magnification ×237. (*From* Konowalchuk J, Speirs JI, Stavrik S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun 1977;18:775–9; with permission.)

is the gold standard for confirming the diagnosis. In addition, pure cultures provide organisms for molecular epidemiology and outbreak investigations by public health laboratories working to prevent transmission in the community.[14,15] With the emerging awareness of STEC non-O157 as diarrheal pathogens, many clinical laboratories now offer immunoassay tests for Shiga toxins or for several non-O157 STEC O-antigens in parallel with stool cultures.[3,7] Immunoassay results can often be provided to the clinician before a final culture result is available. Culture confirmation of STEC should always be attempted by the clinical laboratory, however, or by a reference laboratory to which suspected STEC isolates are sent.[16]

EMERGENCE OF STEC NON-O157 DISEASE

Early recognition that STEC serotypes other than O157 were associated with diarrhea and hemorrhagic colitis prompted development of laboratory testing methods for the detection of now over 150 known STEC non-O157 strains.[3,6] In North America, STEC serotypes O26, O45, O103, O111, and O121 are the most common[3,4]; and in Europe, these serotypes, and O91 and O145, are most frequently isolated from ill patients.[3,4,10] Serotypes of STEC isolated from ruminant reservoir animals and from infected patients vary in prevalence worldwide, indicating a need for clinical laboratories to determine common endemic serotypes while being on the alert for less common or "imported" serotypes in sporadic infections.[1,2,4,17,18] The non-O157 STEC also harbor and express one or both of the Shiga toxin *Stx* genes present on temperate bacteriophages in the STEC genome and, like STEC O157, have caused diarrhea, hemorrhagic colitis, and HUS.[3,4,6,10] Detection of non-O157 STEC in stool cultures is problematic because unlike most O157 STEC, they do ferment sorbitol, and do not grow as sorbitol-negative (nonfermenting) colonies on sorbitol-MacConkey (SMAC) agar that has been so useful for recovery of sorbitol-nonfermenting STEC O157.[3,9]

EMERGING TRENDS IN STEC DISEASE

The severity and long-term sequelae associated with STEC disease warrant a careful consideration of how to improve patient outcome.[17,19] Awareness that STEC non-O157 serotypes are capable of causing postdiarrheal HUS is well established,[3,4,7] but the recovery of diverse serotypes by most clinical laboratories is difficult and can result in delays in providing useful information to clinicians. DNA amplification methods can be used to identify STEC independent of serotype, if genes encoding Stx1 or Stx2 are present.[20] This can be done from isolated colonies, from mixed growth on agar plates, or directly from stool specimens. Commercial kits for real-time amplification tests for Shiga toxin genes, using instrument platforms currently in use in clinical laboratories, may eventually be available.[21]

Sorbitol-fermenting STEC O157, the so-called "SF STEC," are typically nonmotile (H-) STEC O157 first identified in a 1988 outbreak of HUS in Germany.[22] These pathogens present identification challenges analogous to those of non-O157 STEC. The epidemiology of SF STEC O157:H- is interesting because of its geographic clustering in Europe.[20,22]

Loss of the genes that encode Shiga toxins during infection has recently been observed for patients with HUS following STEC diarrhea. Strains of *E coli* having typical STEC serotypes (eg, O26, O111, O103, O121, O145, and O157) but which lack genes for Stx1 and Stx2 have been isolated from stool specimens of HUS patients.[23] Assuming that these strains of *E coli* were the cause of HUS for these patients, the Shiga toxin genes were apparently lost during the course of the infection. This situation potentially renders ineffectual current Shiga toxin immunoassays and

Stx gene amplification testing of bacterial isolates from diarrheal stool specimens in search of STEC. Culturing early in the course of clinical illness,[13] and successive culturing, may allow recovery of toxin-producing STEC before the Shiga toxin genes are lost.[13]

MICROBIOLOGY

Bacterial Physiology and Genetics of STEC

Escherichia coli is the predominant gram-negative facultative anaerobe found as usual intestinal flora in warm-blooded animals, including humans, although it is outnumbered in the intestine by obligate anaerobes, such as *Bacteroides* spp.[24] *E coli* is typically motile by peritrichous flagella, the location of the *E coli* H-antigen, of which over 50 serotypes are known. Some commensal *E coli* may possess a capsule, the site of the *E coli* K-antigen, which serves as a virulence factor for extraintestinal colonization, urinary tract infections, and invasive disease. Over 80 serologically distinct K-antigen specificities are known.[24]

Enterobacteriaceae, the taxonomic family to which *E coli* belongs, includes opportunistic commensal genera, such as *Citrobacter*, *Enterobacter*, *Klebsiella*, and *Proteus*, and noncommensal human pathogens, such as *Salmonella*, *Shigella*, and *Yersinia*.[3,24] The gram-negative cell wall of the Enterobacteriaceae is characterized by the presence of a lipopolysaccharide that is the location of the O-antigen. The O-antigen is a polysaccharide composed of repeating monosaccharide trimers in diverse combinations and sequences. The O-antigen is anchored in the cell wall's outer membrane by a lipid moiety, Lipid A, by an oligosaccharide core (**Fig. 3**). The complex structure of these lipopolysaccharide polysaccharides generates the 100 to 200 distinct *E coli* O-antigen serotypes. Diarrheogenic *E coli* (STEC, ETEC, EIEC, and EPEC) (see **Table 1**) have a relatively restricted number of O-antigen serotypes,[3] and STEC is unique among these in its ability to produce Shiga toxins.

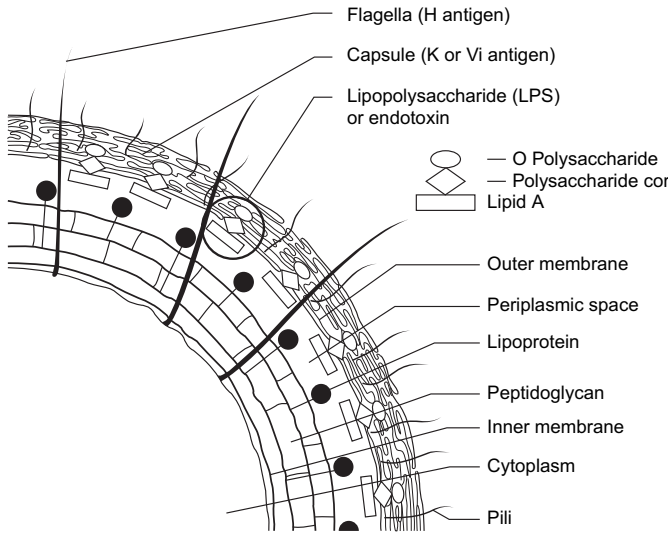

Fig. 3. Antigenic structure of Enterobacteriaceae. (*From* Murray PR, Rosenthal KS, Kabayashi S, et al. Enterobacteriaceae. In: Medical microbiology. 4th edition. Philadelphia: Elsevier Mosby; 2002. p. 267; with permission.)

The STEC O157 chromosome is a closed circular, double-stranded DNA molecule about 5.5 megabases in size.[11,25] **Fig. 4** provides a graphic representation of the STEC O157 chromosome from the strain responsible for the 1996 outbreak in Sakai City, Japan, derived from genome sequencing. The fourth circle of the figure indicates in black the locations of the temperate bacteriophage genomes integrated into the *E coli* chromosome that harbor the genes encoding Stx1 and Stx2. These bacteriophages account for some of the differences evident between the Sakai STEC O157 genome and the genome of a nonpathogenic laboratory strain of *E coli*. Other areas of difference illustrated in **Fig. 4** reflect virulence genes present in STEC O157 but not in a nonpathogenic *E coli*.[11,25]

The integrated, temperate bacteriophage genomes carrying the Shiga toxin genes can be induced to replicate lytically and generate bacteriophage progeny by exposure of STEC to chemical agents or ultraviolet light. These bacteriophages are lambdoid in morphology, with hexagonal heads and long tails.[26] They may be responsible for transmission of Shiga toxin genes between different strains of *E coli* in the gastrointestinal tract, and even toxin gene transfer to other genera and species of bacteria, such as *Citrobacter* spp, *Aeromonas* spp, and *Enterobacter* spp, in which Shiga toxin genes have been reported to occur.[12]

Diagnostic Microbiology of STEC

In the clinical microbiology laboratory, recovery of STEC O157 from stool specimens has been facilitated by the inability of most STEC O157 to ferment sorbitol.[3,9] Because of the worldwide occurrence of this pathogen and its well-documented ability to cause

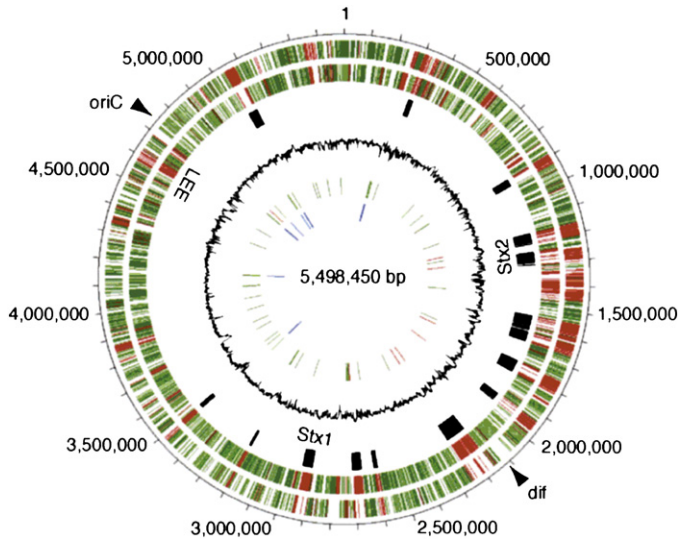

Fig. 4. Circular representation of the *E coli* O157 chromosome. The outermost circle indicates locations on the 5.5-MB genome. The second and third circles show in red the predicted genes in O157 that differ from those of a nonpathogenic laboratory strain of *E coli*. The fourth circle indicates in black the locations of the integrated temperate bacteriophage genomes encoding Stx1 and Stx2 in the O157 chromosome. (*From* Hayashi T, Makino K, Ohnishi M, et al. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res 2001;8:13; with permission.)

serious disease, media chosen for bacterial cultures of all stools from patients with diarrhea or HUS should include SMAC agar for recovery and isolation of STEC O157. SMAC agar containing cefixime and potassium tellurite has been used to suppress interfering gram-negative bacterial growth when isolating STEC O157, but these agents prevent growth of some STEC and are not recommended for routine stool cultures.[3]

Chromogenic agars (eg, CHROMAgar, Becton Dickinson, Sparks, MD, USA) are selective differential media for the visual identification of specific pathogens based on their biochemical phenotypes. Chromogenic O157 agar, like SMAC agar, can provide the laboratory with isolated colonies after overnight incubation for presumptive visual identification as STEC O157, and facilitate prompt confirmation of suspect colonies as STEC O157:H7 by subculture and latex agglutination for O157 and H7 antigens, or for Shiga toxins.[3] Alternatively, ''sweeps'' of confluent growth from a plate with suspect STEC colonies can be tested for Shiga toxin or O-antigens using a commercial immunoassay for which this specimen source is approved according to the package insert.

Recovery of STEC non-O157 from stool specimens of patients with diarrhea is challenging. These pathogens ferment sorbitol and are not detected on SMAC or current chromogenic agars. The importance of testing for non-O157 STEC cannot be overemphasized, however, because at least one third of STEC isolated from ill patients are non-O157 serotypes.[3,4,27,28] Immunoassays from several manufacturers are available to test patient specimens or cultures for STEC.[29]

Direct testing of fresh stool specimens for Shiga toxins by immunoassay before culture can provide the clinician with a qualitative result in 20 minutes to 2 hours, depending on the type of test used. Only immunoassays specifying fresh stool as an acceptable specimen in the package insert should be used for direct ''point-of-care'' Shiga toxin testing. An optical immunoassay (eg, Biostar OIA SHIGATOX, Inverness Medical Professional Diagnostics, Princeton, NJ, USA) is available for direct testing of stool specimens, providing a toxin result in 20 minutes, without differentiation of Stx1 and Stx2.[28] A microwell plate immunoassay (eg, Premier EHEC, Meridian Bioscience, Cincinnati, OH, USA) for direct testing of stool specimens provides toxin results in about 2 hours. The sensitivity of these direct tests is good, between 80% and 100%, and specificity is 99%.[28] A greater variety of immunoassays is available for detection of Shiga toxins and O-antigens in growth from overnight (18–24 hours) cultures, either in broth or on agar media. If no toxins are detected in direct testing of stool specimen, a Shiga toxin immunoassay can be repeated from overnight growth, usually with greater sensitivity than can be obtained from direct testing of stool specimens.[27,28]

One approach is to sample ''sweeps'' of mixed colonial growth from SMAC or other agar media after overnight incubation. Individual colonies of potential STEC growing on SMAC or other enteric culture plates may be picked for identification by serotyping or for Shiga toxin immunoassay.[3]

Suspensions of the sweeps or colonies can be tested for Shiga toxins using an optical immunoassay (eg, Biostar OIA SHIGATOX),[28] a lateral flow immunoassay (eg, Meridian Immunocard STAT!EHEC, Meridian Bioscience, Cincinnati, OH, USA) (**Fig. 5**), or an enzyme immunoassay (eg, ProSpect Shiga toxin *Escherichia coli* Microplate Assay, Alexon-Trend, Ramsey, MI, USA).[27] Rapid tests (optical and lateral flow immunoassays) provide a qualitative Shiga toxin result in 10 to 15 minutes and microplate assays in about 2 hours. The sensitivity and specificity of immunoassays should not be assumed to be 100%, so results must be interpreted cautiously until STEC colonies are isolated and identified. Real-time polymerase chain reaction testing of stool specimens directly, or of overnight cultures, can also provide Stx1 or Stx2

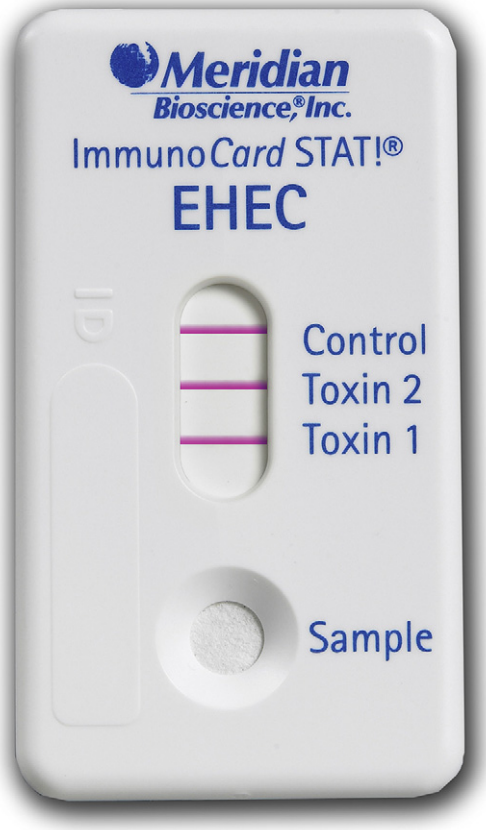

Fig. 5. Shiga toxin lateral-flow immunoassay device for testing broth and agar cultures. This device distinguishes Stx1 from Stx2. (*Courtesy of* Meridian Bioscience, Cincinnati, OH.)

gene results to clinicians in 30 to 60 minutes when available for clinical laboratories.[20,21] As with immunoassay results, molecular testing results must be confirmed by isolation of STEC from stool and culture.

Many STEC express a distinctive hemolytic phenotype on enterohemolysin (Ehly) agar (eg, Sifin, Berlin, Germany).[3,10,30] If this agar is available, individual colonies from Ehly agar can be picked and tested by latex agglutination for common STEC O-antigen serotypes, such as O157, O26, and O111, or for Shiga toxins by immunoassay.[3,6]

Immunomagnetic separation is used in the food industry for enhancing the recovery of diverse STEC serotypes.[3] It could be used on a research use only basis for the enrichment of stool specimens or broth cultures for STEC by incubation with sterile magnetic beads to which selected STEC O-antigen–specific antibody molecules have been attached. Following incubation the beads are aseptically rinsed and cultured, and colonies or mixed growth tested as described previously. Reagents for immunomagnetic separation are commercially available for several STEC (eg, O157, O111, O26, from Denka Seiken, Japan; O157, from Invitrogen Dynal, Oslo, Norway). Beads for immunomagnetic separation could be custom-requested from manufacturers by laboratories based on local STEC serotype prevalence in patients and animal reservoirs.[1–4]

Table 2
Recent STEC outbreaks, 2006–2009

Country and Year of Outbreak	STEC Serotype	Transmission Source or Vehicle	Cases/Hosp/HUS/ Deaths[a]	Action Taken	Text References
United Kingdom, Wales, 2009	O157:H7	Fast food outlet	4/2/2/0	Analysis of isolates by PT, PFGE, VNTR; active case finding by local practitioners	33
United Kingdom, Wales, 2009	O157:H7	Dance camp; campsites on farms with animals; unchlorinated water being investigated	2/–/–/–	Contacts sought by social network Web sites, telephone, e-mail	34
United States, 2009	O157:H7	Refrigerated cookie dough, uncooked	72/34/10/0	Recall of product by manufacturer	35
Netherlands, 2008–2009	O157:H- (nonmotile)	Raw minced beef	20/7/0/0	Traceback investigation	36
United States, 2008	O157:H7	Commercial ground (minced) beef	49/27/1/0	Beef product recall (5.3 million pounds)	37
Canada, 2008	O157:H7	Raw onions	235/26/1/0	Traceback investigation	38
Netherlands and Iceland, 2007	O157:H-	Lettuce processed at Dutch plant	Netherlands: 41/0/0/0 Iceland: 9/0/0/0	Traceback investigation	39
Scotland, 2007	O157:H7	Cold meat salad	9/2/0/0	Public notified; hotel kitchen closed and cleaned; disinfectant washing procedure instituted	40
United States, 2007	O157:H-	Petting zoo (goats, sheep, llama)	7/2/0/0	Case finding among staff, visitors; zoo closed; animals tested for colonization	5

United States, 2006	O157:H7	Spinach	199/102/31/3	Public warning; product recall; PFGE analysis of outbreak strains	[41]
Norway, 2006	O103:H25 (notable for loss of Stx2 gene during infection)	Cured mutton sausage	17/–/10/1	Public warning; product recall; sheep slaughter changes implemented	[42]
Japan, 2006	O26	Nursery school; person-to-person spread from index patient inferred	26/0/0/0	Case finding; PFGE analysis of isolates	[43]
Japan, 2006	O103	Nursery school; person-to-person spread from index patient inferred	8/0/0/0	Case finding; PFGE analysis of isolates	[44]

Abbreviations: HUS, hemolytic uremic syndrome; PFGE, pulsed-field gel electrophoresis; PT, phage typing; VNTR, variable number of tandem repeats.
[a] Numbers of: No. of Cases/No. hospitalized/No. with HUS/No. deaths.
Data from Refs. [5,33–44]

EPIDEMIOLOGY

STEC infection is a zoonotic disease, for which small and large ruminant animals, notably cattle, are the natural reservoir, harboring STEC as normal gastrointestinal flora.[1,2] Transmission to humans commonly occurs through consumption of STEC-contaminated raw or undercooked meat, or of produce contaminated with cattle feces through farming or production practices. The infectious dose of STEC is low, about 100 bacteria,[3] so person-to-person transmission can occur, leading to secondary cases in contacts of infected persons. Clinically recognized STEC diarrhea, and probably many subclinical STEC infections, occurs through direct animal-to-human contact.[5] Risk of transmission to humans from food sources can be substantially reduced by careful washing of produce; pasteurization of juices and milk; and cooking to allow the internal temperature of meat, especially ground (minced) beef, to reach 70°C (160°F).[4]

Approximately 75% of cases of HUS occur in children, following diarrhea caused by an STEC infection. HUS is a significant cause of acute renal failure in children.[9,19] In the United States, approximately 90% of HUS cases are caused by STEC O157:H7, but only 50% or less in other countries.[3,4,9,13] Children under 10 years of age are at greatest risk for serious STEC infections. Approximately 15% of children with STEC diarrhea develop HUS.[9,31] Half of these require dialysis for renal failure, and the HUS case-fatality rate is approximately 5%.[4,9,19] The incidence of HUS worldwide varies widely. In Argentina, the incidence is 12 per 100,000 children under 5 years of age,[32] but can be 10-fold lower elsewhere in the world.[9]

The worldwide distribution and diversity of STEC serotypes in recent outbreaks is evident from **Table 2**. Several interactive sources for tracking STEC disease and serotypes worldwide are accessible electronically. Data for STEC from 36 reporting countries are available from Enter-Net, funded by the European Center for Disease Control (http://ecdpc.europa.eu/documents/ENTER_NET/vtec07q2.pdf; accessed August 5, 2009).

Similar data, both in tabular and map format, are available for the United States in publications from the Centers for Disease Control and Prevention.[45] The World Health Organization Weekly Epidemiologic Record indexes "VTEC," with fewer entries for "STEC," at www.who.int/wer/en (accessed June 10, 2009). The World Health Organization provides interactive map building capability for the World Health Organization European Region through its Computerized Information System for Infectious Disease, accessible through www.who.int. For STEC, the Computerized Information System for Infectious Disease map query-builder uses "6080" as the reference number for generating distribution maps of "enterohemorrhagic *E coli*" (accessed June 10, 2009).

International interactive maps of disease occurrence are generated by ProMED, a global reporting system of emerging infectious diseases, at http://www.promedmail.org/. The maps are accessible at www.healthmap.org. A healthmap.org map locating current STEC outbreaks in the United Kingdom and Wales[33,34] is shown in **Fig. 6**.

Statutory reporting of STEC infections has evolved with the increasing recognition of serious disease from non-O157 STEC infections. Currently in the United States, STEC infections of all serotypes are reportable to the National Notifiable Diseases Surveillance System of the Centers for Disease Control and Prevention.[45] Molecular techniques, such as phage typing and pulsed-field gel electrophoresis, are useful for comparing STEC isolates from outbreaks to link ill patients with contacts and with potential sources of infection. Databases are available for using these techniques to compare newly isolated outbreak strains for tracking the sources of outbreaks.[14,15]

Fig. 6. Interactive map showing location in United Kingdom and Wales of two STEC O157:H7 outbreaks, July, 2009. (*From* ProMED. PRO/AH/EDR>E. COLI O157-UK: Wales dance camp, alert 17-Aug-2009. Archive Number 20090817.2915. Available at: http://www.promedmail.org and www.healthmap.org. Accessed August 18, 2009; Brownstein JS, Freifeld CC, Reis BY, et al. Surveillance sans frontières: internet-based emerging infectious disease intelligence and the healthmap project. PLoS Med 2008;5:e151; with permission.)

CLINICAL PRESENTATION

The clinical presentation of STEC infection is diarrhea, consisting of more than three unformed stools in a 24-hour period with or without blood.[5] The diarrhea may be intermittent, watery, or nonwatery, and may be associated with dehydration consistent with the fluid loss. Other symptoms often associated with STEC diarrhea include abdominal cramping, nausea, headache, vomiting, and fever.[3,4,7,9,19]

Based on experience with STEC O157 in children, diarrhea, abdominal pain, painful defecation, vomiting, and fever occur about 3 days after ingestion of an infectious dose of bacteria. Bloody diarrhea develops in 90% of children after another 3 days. The diarrhea symptoms abate about 7 days after onset. About 85% of children recover spontaneously, with 15% developing HUS and at risk of death (5% mortality).[9]

The clinical presentation of HUS in a patient with prior gastrointestinal or influenza-like symptoms is usually evidence of bleeding, either in vomitus or stool; severe oliguria; hematuria; microangiopathic hemolytic anemia; hypotension; and perhaps neurologic changes. Because of the risk of HUS, renal failure, and death in patients with intestinal STEC infections, especially in children, it is extremely important to assess renal function of the patient at presentation, which along with dehydration, may necessitate emergent care. The question of whether or not to treat a diarrheal infection caused by STEC must be considered promptly because of the generally accepted risk of increasing the severity of diarrhea and HUS caused by STEC if antimicrobial agents are given to STEC-infected patients.[4,9,19,31]

PATHOGENESIS

Ingested STEC reach the small intestine and the colon and multiply there in competition with the normal bacterial flora. A number of bacterial structures (fimbriae, pili) and adhesion molecules (adhesins) are thought to mediate adherence of STEC to the intestinal epithelial cells, allowing the Shiga toxins secreted by STEC to interact with the enterocyte plasma membrane surface.[23,46–48]

The STEC secrete Shiga toxins Stx 1 and Stx2, which bind to the enterocytes, the absorptive epithelial cells present on the luminal surface of the small and large intestines (**Fig. 7**). Stx1 and Stx2 are exotoxins of the AB5 class of toxins.[12] **Fig. 8** is a ''ribbon diagram'' illustrating the three-dimensional structure of the A and B polypeptides of Stx2 based on radiograph crystallography.[49,50] The pentameric B portion of the toxin, consisting of five identical B polypeptides, binds to the cellular glycolipid receptor globotriaosylceramide (Gb3) present on the plasma membrane of enterocytes and other cells. The monomeric A subunit of the Shiga toxin then enters the enterocyte by endocytosis and is transported to the rough endoplasmic reticulum by the Golgi apparatus.[12] The A subunit is proteolytically cleaved in the cell cytoplasm, liberating the N-terminal (A1) portion, a glycosidase that hydrolyzes a specific adenine-ribose bond in the ribosomal 28S RNA. This cleavage prevents aminoacyl-tRNA binding, and irreversibly inhibits protein synthesis, resulting in cell death (see **Fig. 2**).[48]

Once the Shiga toxins enter the bloodstream by the damaged intestinal epithelium, the precise mechanism of which is still unclear, capillary endothelial cells are exposed to the Shiga toxin and killed by the same mechanism. The endothelial cell lysis is accompanied by platelet activation and aggregation, leukocyte adherence, cytokine secretion, and vasoconstriction, contributing to fibrin deposition and clot formation within the capillary lumen and in the subendothelial tissue.[17,46,47] This thrombotic microangiopathy then occurs distally as the Shiga toxins are carried by the bloodstream

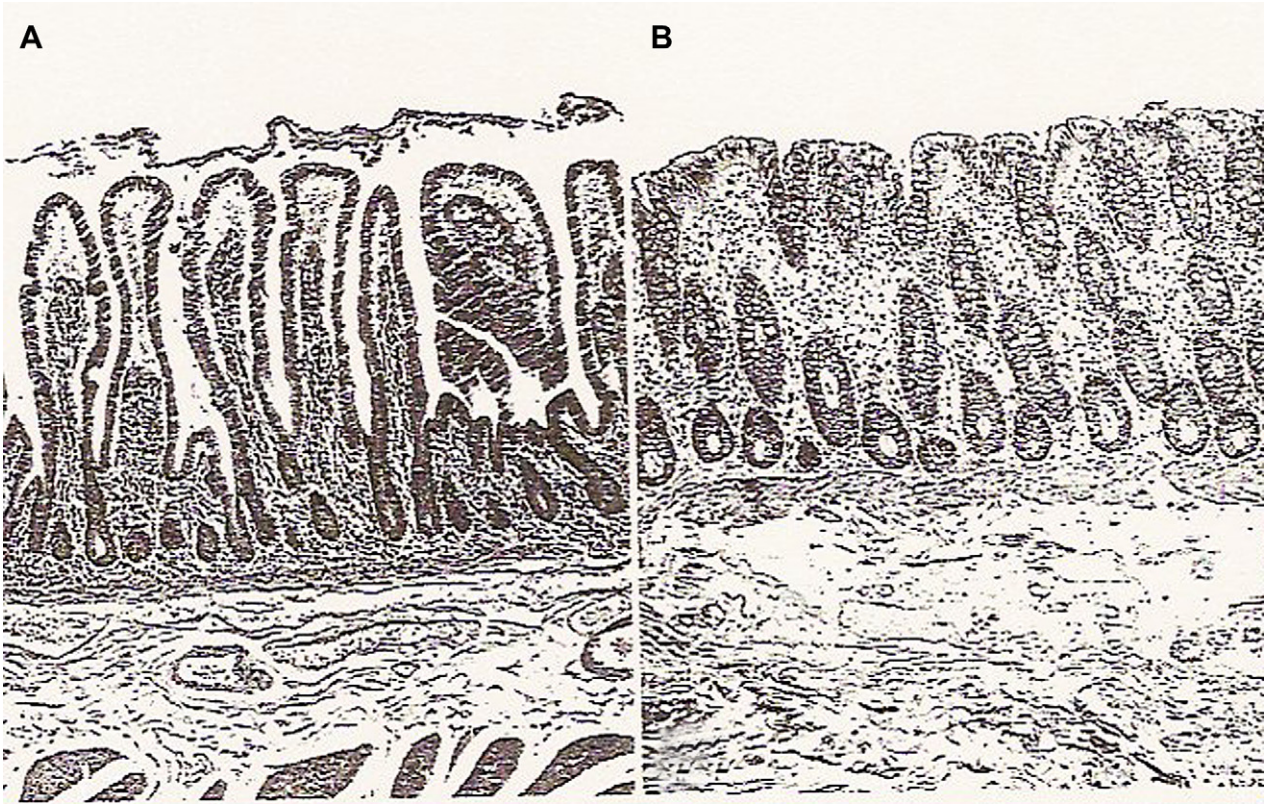

Fig. 7. Normal intestinal mucosa. (*A*) Luminal surface of the normal small intestine, with enterocytes lining the villi and intestinal crypts. (*B*) Normal colon histology showing colonic crypts and a flat mucosal surface lined with enterocytes. The enterocytes are the target of STEC Shiga toxins. H&E staining. Magnification ×70 approximately. (*From* Chen L, Crawford JM. The gastrointestinal tract. In: Kumar V, Abbas AK, Fausto N, editors. Robbins and Cotran pathologic basis of disease. 7th edition. Philadelphia: Elsevier Saunders; 2005. p. 828; with permission.)

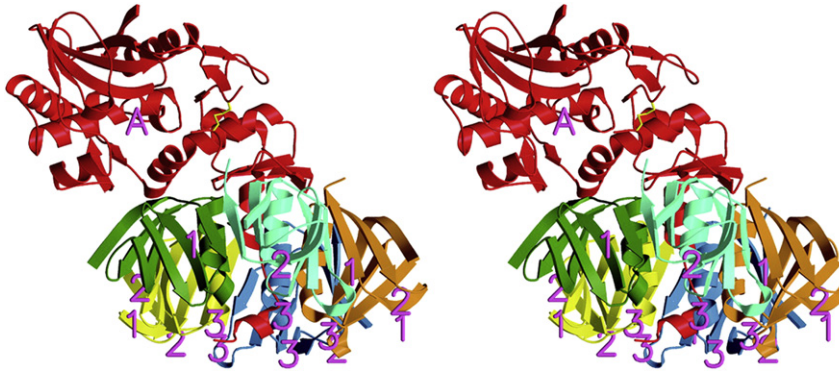

Fig. 8. Ribbon diagram of Stx2 from *E coli* O157:H7. Shiga toxin is a class AB5 toxin. The monomeric A polypeptide is red. The five B polypeptides are orange, cyan, green, yellow, and blue. Binding of the pentameric B portion of the toxin to the cell surface allows entry of the A subunit into the cell, where it functions enzymatically to stop protein synthesis and kill enterocytes and other cells to which the Shiga toxin binds. (*From* Fraser ME, Fujinaga M, Cherney MM, et al. Structure of Shiga toxin type 2 (Stx2) from *Escherichia coli* O157:H7. J Biol Chem 2004;279:27513; with permission.)

to the kidneys, resulting in fibrin deposition in glomerular capillaries, hematuria, and renal failure (**Fig. 9**).[9,31,47]

DIAGNOSIS

The clinical presentation of STEC infection is diarrhea, with or without blood present, which may be intermittent, watery, or nonwatery, often accompanied with abdominal cramping, nausea, headache, vomiting, and fever.[4,7,9,51]

Fig. 9. Fibrin stain showing platelet-fibrin thrombi (*dark areas*) in the glomerular capillaries. Glomerular injury is characteristic of microangiopathic disorders, such as hemolytic uremic syndrome (HUS). Fibrin stain. Magnification ×240 approximately. (*From* Alpers CE. The kidney. In: Kumar V, Abbas AK, Fausto N, editors. Robbins and Cotran pathologic basis of disease. 7th edition. Philadelphia: Elsevier Saunders; 2005. p. 1010; with permission.)

Laboratory diagnosis of STEC infection in a patient with diarrhea is based on microscopic examination of stool when appropriate (eg, to exclude parasites and to visualize *Vibrio* and *Campylobacter* spp)[7]; bacterial stool cultures; detection of Shiga toxin by immunoassay; and detection of genes encoding Shiga toxin by DNA amplification. Confirmation of STEC as the etiologic agent of diarrhea requires the recovery and isolation in pure culture of STEC from a patient's stool specimen.[3]

Stool specimens should be obtained for culture as early as possible from the patient presenting with diarrhea, and specimens for culture collected successively if no pathogen is initially identified. In patients who develop HUS, STEC may be unrecoverable by the time HUS develops.[13] Ideally, specimens for toxin or O-antigen immunoassay, or DNA amplification, should be the same ones collected for cultures. These tests may provide preliminary information useful to clinicians in advance of a confirmatory culture result that requires 1 or more days to obtain. Any report from the laboratory stating that STEC O157 specifically was not recovered or detected should include a comment that STEC non-O157, such as STEC O26 and O111, can also cause diarrhea and HUS and should be considered as potential etiologies.[3,4] This reporting by the laboratory allows the clinician to request additional testing if it seems indicated.

STEC isolates recovered by the clinical laboratory, and cultures of any presumptive STEC identified by toxin immunoassay or other test, should be submitted to a local or national laboratory for confirmatory testing and outbreak investigation if appropriate.[3,14,15,45]

DIFFERENTIAL DIAGNOSIS

A patient presenting with diarrhea must be assessed not only for infection with gastrointestinal pathogens, but also for other illnesses and conditions with associated diarrhea, such as Legionnaire's disease, severe acute respiratory syndrome, influenza, and chemical and physical injury.[7,51]

Table 3 summarizes 23 causes of diarrhea, including STEC, and provides information useful in formulating a broad differential diagnosis, which can then be refined based on the patient's current illness course and exposure history (food consumption, contact with other ill persons, animals, fomites, social settings). An appreciation of this broad differential is essential to identify the most immediately life-threatening etiologies in a patient and begin a treatment plan without delay.

TREATMENT, PROGNOSIS, AND LONG-TERM OUTCOME

The treatment of patients with STEC diarrhea consists of fluid replacement, supportive care, and careful monitoring of kidney function, without antibiotic therapy.[4,31] Close attention must be given to the possible development of HUS or other associated thrombotic microangiopathy-associated conditions, such as hemorrhagic colitis or thrombotic thrombocytopenic purpura.[9,13,47] HUS can develop in patients of all ages, so vigilance must extend across the age spectrum.[13,47] Renal failure can usually be managed by dialysis and patients usually recover in several weeks.[13,31] Treatment of STEC infections with antimicrobial agents is not recommended because of studies demonstrating ineffectiveness and even potential harm to patients who receive antibiotics for STEC diarrhea.[19,31] Long-term follow-up studies to determine the outcome of patients who have experienced STEC diarrhea-associated HUS indicate that there is some increase in risk of renal impairment and hypertension in these patients.[19,31,47,48,52]

Table 3
Differential diagnosis of diarrhea in patients with suspected STEC

Pathogen	Incubation Period	Blood in Stool	Abdominal Cramping and Pain	Nausea or Vomiting	Fever	Other Signs and Diagnostic Aids	Diagnostic Laboratory Testing
Bacillus anthracis (gastrointestinal anthrax)	2 d to wk	+	+	+	+	Patient history: exposure may be accidental from consumption of infected meat	Culture of food, any lesions, blood; CDC select agent; culture hazard
Brucella spp	7–21 d	+	–	–	+	Patient history: bacteremia and fever; unpasteurized dairy product consumption; travel to endemic area; muscle and joint pain; headache	Blood cultures with special incubation request; CDC select agent; culture hazard
Campylobacter spp	2–5 d	+/–	+	+	+	Consumption of or contact with raw or undercooked poultry	Stool culture (special request)
Clostridium difficile (*C difficile* associated disease); Community-associated *C difficile* associated disease	Variable (d to mo)	+/–	+	+/–	+	May occur in patients with or without prior antibiotic use	Stool toxin EIA; anaerobic stool culture and toxin EIA of isolates
Cryptosporidium spp	2–28 d	–	+	+	+	May be chronic in ICH	Microscopic stool examination; DFA; EIA

Cyclospora spp	1–11 d	–	+	+	–	Fatigue; may be chronic if unrecognized	Microscopic stool examination
EIEC	10–18 h	– early; <10% + late	+	–	+	Fecal leukocytes late, as seen with *Shigella* spp	Stool culture for other pathogens
Entamoeba histolytica	2 d–4 wk	+	+	–	+	Invasive; liver abscess if chronic; may be confused with STEC; patient food and exposure history very important	Microscopic stool examination; serology for chronic or invasive disease; stool EIA or PCR
EPEC	9–12 h	+/–	+	+	+	Infant diarrhea; mucus in stool; dehydration severe; prolonged infections	Stool culture for other pathogens
ETEC	1–3 d	–	+	–	–	Patient history useful (travel, infant weaning)	Stool culture for other pathogens
Giardia spp	1–4 wk	–	+	–	–	Flatulence, bloating; may be chronic if unrecognized	O&P stool examination; stool DFA or EIA
Norovirus	24–48 h	–	–	+	–	Outbreak settings common; most common viral cause of gastroenteritis	PCR testing of stool
Rotavirus	1–3 d	–	–	+	+/–	Common in children; outbreak settings	Stool EIA for rotavirus

(*continued on next page*)

Table 3
(*continued*)

Pathogen	Incubation Period	Blood in Stool	Abdominal Cramping and Pain	Nausea or Vomiting	Fever	Other Signs and Diagnostic Aids	Diagnostic Laboratory Testing
Salmonella enterica subsp. enterica; 2500 serovars; nontyphoidal	1–3 d	–	+/–	+	+	May be chronic if not recognized or untreated	Stool culture
Shigella spp	1–2 d	+	+	+	+	*S dysenteriae* serotype 1 especially severe due to Shiga toxin; all *Shigella* are invasive of intestinal epithelium	Stool culture (special request for *S dysenteriae* serotype 1)
STEC, O157 or non-O157 (EHEC, VTEC)	1–8 d	+/–	+	+	+/–	Nonbloody diarrhea may precede blood in stool; oliguria, renal failure, hemolytic uremic syndrome; history of undercooked beef consumption	Stool culture to include SMAC agar for O157 STEC (special request); Shiga toxin EIA; O157 EIA; PCR testing of stool for Stx genes
Toxins, bacterial, preformed: *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus aureus*	1–16 h Bc; 12–72 h Cb; 8–16 h Cp; 1–6 h Sa	–	+	+	–/+	Sudden onset vomiting with Sa; diplopia and muscle paralysis with Cb; Cb is life-threatening	Toxin testing of food

Toxins: fish, shellfish, and mushrooms	<30 min to 8 h	–	+	+	+/–	Visual disturbance, confusion, numbness, altered sensations; may be life-threatening	Toxin testing of food
Toxins, chemical: organic compounds, metals (As, Sn, Tl, Zn), nitrite, fluoride	5 min to 8 h	–	+	+	–	Headache, nervousness, twitching movements, visual disturbance	Toxin testing of food
Trichinella spp	1 d–8 wk	–	+	+	+	Myalgias, periorbital edema; cardiac and neurological involvement possible	Larval cysts detectable in muscle tissue by microscopy
Vibrio cholerae, serogroup 01 or 0139	1–3 d	–	–	+	–	Profuse watery diarrhea; dehydration life-threatening	Stool culture (special request)
Vibrio parahemolyticus, *V mimicus*, *V fluvialis*, *V furnissii*, *V hollisae*	2–48 h	+/–	+	+	+	Patient history of seafood consumption	Stool culture (special request)
Yersinia enterocolitica and pseudotuberculosis	24–48 h	+/–	+/–	+	+	Mesenteric lymphadenitis mimicking appendicitis	Stool culture (special request)

Symbols used: + usually present; +/– may be present; – rarely present.

Abbreviations: DFA, direct fluorescent antibody staining; EIA, enzyme immunoassay; ICH, immunocompromised host; Bc Cb Cp Sa (for toxins, bacterial, preformed) are *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, and *Staphylococcus aureus*, respectively; O&P, ova and parasite; PCR, polymerase chain reaction; SMAC, sorbitol-Mac Conkey.

Adapted from Centers for Disease Control and Prevention. Diagnosis and management of foodborne illnesses: a primer for physicians and other health care professionals. MMWR Morb Mortal Wkly Res 2004;53:1–33.

Additional data from Refs. [3,4,51]

SUMMARY

STEC (VTEC, EHEC) are important enteric pathogens worldwide, causing diarrhea with or without blood visibly present, and HUS. Children under the age of 10 years are at greatest risk. In children with STEC diarrhea, 15% develop HUS, which has 5% mortality rate. The STEC are unique among diarrheogenic *E coli* in producing Shiga toxin type 1 and type 2, the virulence factors responsible for bloody diarrhea and HUS. Cattle and other ruminants are the natural reservoir of STEC as their normal intestinal flora. Humans become infected by consumption of foods contaminated with cattle feces, notably undercooked ground (minced) beef, nonpasteurized products, and leafy vegetables that are consumed without cooking. The O157:H7 serotype of STEC predominates in human infections, and has been associated with outbreaks of diarrhea and HUS, but non-O157 STEC currently cause at least one third of STEC diarrhea and HUS. Diagnosis of STEC infection and of HUS is based on clinical signs; patient history; monitoring of renal function (especially in children); rapid testing of stool specimens for Shiga toxins; and isolation of STEC from stool cultures. Early diagnosis of STEC infection is important because of the contraindication for treating STEC using antimicrobial agents, and the intense supportive care needed if renal failure occurs.

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