Enterohemorrhagic Escherichia coli (EHEC)

1. Organism

Like generic E. coli, toxin-producing Shiga-toxigenic Escherichia coli (STEC) are Gram-negative, rod-shaped bacteria, but are characterized by the production of Shiga toxins (Stx). Depending on the reference cited, there are 200 to 400 STEC serotypes, many of which have not been implicated in human illness; however, a subset of STEC called enterohemorrhagic Escherichia coli (EHEC), the topic of this chapter, includes only those that cause serious illness. Serotype O157:H7 is the prototypic EHEC strain.

Although O157:H7 is currently the predominant strain and accounts for ~75% of the EHEC infections worldwide, other non-O157 EHEC serotypes are emerging as a cause of foodborne illnesses. In the United States a group often referred to as the “big 6” (O111, O26, O121, O103, O145, and O45) accounts for the majority of the non-O157:H7 serotypes isolated from clinical infections and, therefore, is currently a focus of concern. However, other EHEC serotypes, such as O113, O91, and others, also can cause severe illness. A recent example is the large outbreak, in 2011, that was centered in Germany, but also affected various other countries in the European Union. The pathogen was identified as an E. coli strain of serotype O104:H4 that produced Shiga toxin and, therefore, was thought to be an EHEC. However, genetic analysis showed that this pathogen had 93% genetic homology with a strain of Enteroaggregative E. coli (EAEC), which is known for causing persistent diarrhea in underdeveloped countries, but has seldom been implicated in major foodborne incidents. Hence, the O104:H4 strain that caused the outbreak appears to be an EAEC strain that acquired the ability to produce Shiga toxin.

For Consumers: A Snapshot

Most E. coli bacteria are harmless, but some produce a toxin (Shiga toxin) that can cause serious illness, including bloody diarrhea, blood-clotting problems, kidney failure, and death. Not all of the Shiga-producing E. coli can cause these problems, but the subset called enterohemorrhagic E. coli (EHEC) can. You might have heard news reports about these EHEC bacteria, such as E. coli O157:H7, when they’ve caused outbreaks of foodborne illness. EHEC outbreaks have been traced to many kinds of foods; for example, ground meats, unpasteurized (“raw”) milk, unpasteurized fruit juice, lettuce, spinach, sprouts, and, more recently, commercially manufactured frozen cookie dough. Some people get the less serious form of the infection, which can range from no symptoms to diarrhea that starts out watery, then turns bloody. But the infection sometimes progresses into the life-threatening form of the illness that causes kidney failure and other problems, with children and people with weak immune systems being at especially high risk. Cooking ground beef well; washing raw fruits and vegetables under clean, running water; and not drinking unpasteurized (“raw”) milk or eating certain cheeses made from it are some of the things you can do to help protect yourself.
Currently, it is difficult to determine which serotypes of \textit{E. coli} are EHEC and equally challenging to predict the emergence of strains that can acquire the genes for Shiga toxin production or other virulence factors and so cause human illness. EHEC are characterized by:

- production of Stx, including Stx1 and/or Stx2. Stx1 is nearly identical to the toxin produced by \textit{Shigella dysenteriae} Type I. There are many subtypes of both toxins, and some subtypes of Stx2 appear to be implicated in human illness. Stx2 is most often associated with severe sequelae, such as hemolytic uremic syndrome (HUS), which is characterized by acute renal failure.
- presence of LEE (“locus for enterocyte effacement”; pathogenicity island that encodes for intimin, a protein that enables bacterial attachment to epithelial cells).

There are also several other putative virulence factors, including enterohemolysin, but the role of these factors in pathogenesis remains undetermined.

2. Disease

- **Mortality**: Patients whose illness progresses to HUS have a mortality rate of 3% to 5%.
- **Infective dose**: The infective dose of EHEC O157:H7 is estimated to be very low, in the range of 10 to 100 cells. The infective dose of other EHEC serotypes is suspected to be slightly higher.
- **Onset**: Symptoms usually begin 3 to 4 days after exposure, but the time may range from 1 to 9 days.
- **Disease / complications**: Infections from EHEC may range from asymptomatic-to-mild diarrhea to severe complications. The acute symptoms are called hemorrhagic colitis (HC), characterized by severe abdominal cramps and bloody diarrhea, which may progress to such life-threatening complications as HUS or thrombotic thrombocytopenia purpura (TTP) – conditions that are most often associated with O157:H7, but that also can occur with other EHEC serotypes. About 3% to 7% of HC cases progress to HUS or TTP.

Some evidence suggests that Stx2 and intimin are associated with progression to severe disease, such as HUS. Kidney cells have a high concentration of Stx receptors; hence, the kidney is a common site of damage. Some survivors may have permanent disabilities, such as renal insufficiency and neurological deficits.

Antibiotic therapy for EHEC infection has had mixed results and, in some instances, seems to increase the patient’s risk of HUS. One speculation is that antibiotics lyse EHEC cells, releasing more Stx into the host.

- **Symptoms**: Hemorrhagic colitis is characterized by severe cramping (abdominal pain), nausea or vomiting, and diarrhea that initially is watery, but becomes grossly bloody. In some cases, the diarrhea may be extreme, appearing to consist entirely of blood and occurring every 15 to 30 minutes. Fever typically is low-grade or absent.
- **Duration**: In uncomplicated cases, duration of symptoms is 2 to 9 days, with an average of 8 days.
- **Route of entry**: Oral (e.g., ingestion of contaminated food, water, or fecal particles).
• **Pathway**: After ingestion, EHEC attaches to intestinal epithelial cells via LEE-encoded factors and produces Stx that are internalized, activated, and can pass into the bloodstream to become systemic.

3. **Frequency**

There are about 63,000 cases of EHEC infections in the U.S. yearly, according to a report by the Centers for Disease Control and Prevention (CDC). Ground beef and beef products continue to be implicated in most infections; however, contaminated produce increasingly has been implicated as a vehicle. As for STEC non-O157, the CDC estimates that 112,752 cases, per year, are attributed to foodborne illness in the U.S.

EHEC O157:H7 was first identified in an outbreak, in 1982, in which hamburgers from a fast-food restaurant were the vehicle. In 1991, hamburgers from fast-food restaurants were implicated in another outbreak, which affected about 700 people in four states. In the mid 1990s, a large outbreak was traced to unpasteurized juices. The largest O157:H7 outbreak on record took place in Japan; radish sprouts were implicated and about 10,000 people were affected. Since then, O157:H7 has been implicated in numerous outbreaks that involved lettuce, salads, various types of sprouts, and, in 2006, bagged spinach. In 2009, an O157:H7 outbreak in the U.S. was traced to frozen, raw cookie dough.

About a dozen non-O157:H7 EHEC outbreaks have been recorded in the U.S., but incidences may be underestimated due to lack of routine testing and appropriate testing methods.

4. **Sources**

Raw or undercooked ground beef and beef products are the vehicles most often implicated in O157:H7 outbreaks. Earlier outbreaks also implicated consumption of raw milk. O157:H7 can develop acid tolerance, as evidenced by infections in which acid foods (<pH4.6) were implicated, such as yogurt, mayonnaise, fermented sausages, cheeses, and unpasteurized fruit juices.

Various water sources, including potable, well, and recreational water, also have caused EHEC infections, as has contact with animals at farms or petting zoos.

Produce, including bagged lettuce, spinach, and alfalfa sprouts, increasingly is being implicated in O157:H7 infections.

Interestingly, infections in the U.S. by non-O157:H7 EHEC has been caused by many of these same vehicles, but, as of this writing, beef products have seldom been implicated.

Person-to-person transmission of infection is well documented.

Additional information is available from “*Escherichia coli* Serotype O157:H7: Novel Vehicles of Infection and Emergence of Phenotypic Variants,” by Dr. Peter Feng, FDA. *Emerging Infectious Diseases (1995) 1(2)*
5. Diagnosis

Unlike generic *E. coli*, EHEC O157:H7 do not ferment the sugar sorbitol, so an effective method is to plate patient’s bloody diarrhea samples onto sorbitol MacConkey medium to screen for sorbitol non-fermenting isolates. These are then typed serologically using antibodies to the O157 and the H7 antigens. However, as other EHEC serotypes are increasingly causing illness, clinical samples are now simultaneously tested for the presence of Stx using commercially-available antibody kits. Any STEC strains found are then serotyped and identified. There are also many PCR assays specific for Stx genes that may be used for screening clinical samples.

6. Target Populations

All people are believed to be susceptible to hemorrhagic colitis, but young children and the elderly are more susceptible and at higher risk for the illness to progress to more severe complications. Others with weak immune systems also are at risk, such as people with some chronic diseases or AIDS, and people on immunosuppressive medications; for example, some drugs used for arthritis and cancer chemotherapy.

7. Food Analysis

Presence of EHEC O157:H7 in foods can be determined by plating culture enrichment of food samples onto selective and differential media. Unlike typical *E. coli*, O157:H7 do not ferment sorbitol and are negative with the MUG assay, so these tests are commonly used to distinguish O157:H7 strains from other *E. coli* prior to serological testing for the O157 and H7 antigens and also for the presence of Stx genes by PCR. Molecular assays also exist that can specifically detect O157:H7 strains using unique mutational markers.

Detection of non-O157:H7 EHEC, however, is more complex, due to the lack of unique traits. For non-O157 EHEC, food enrichment is first screened for Shiga toxin using an antibody assay or for Stx genes by PCR. Enrichment cultures that are positive for Stx are plated on agar media, and multiple isolates are then tested for Stx genes, in order to obtain a pure culture isolate. These putative STEC isolates are then retested for virulence genes and their serotype determined. This process is both time-consuming and labor-intensive and may require screening hundreds of isolates.

There are numerous commercially-available kits to test for Stx, O157, and a few other EHEC serotypes. However, there are several Stx subtypes and many EHEC serotypes, and not all of these can be detected by commercial test kits. The *Escherichia coli* link to the FDA Bacteriological Analytical Manual, Chapter 4, provides a description of methods to test for common *E. coli*. Methods for EHEC and O157:H7 are described in Chapter 4a.

8. Examples of Outbreaks

For more information about recent outbreaks see the Centers for Disease Control and Prevention (CDC) Morbidity and Mortality Weekly Reports.
9. Other Resources

More information is available from the following sources.

- **USDA (August 11 1998)** – USDA Urges Consumers to Use Food Thermometer When Cooking Ground Beef Patties
- **CDC** – General information about *Escherichia coli O157:H7*
- **Produce Handling and Processing Practices**, from *Emerging Infectious Diseases*, CDC
- **Risk assessment** of *E. coli O157:H7* in ground beef, from the USDA Food Safety and Inspection Service