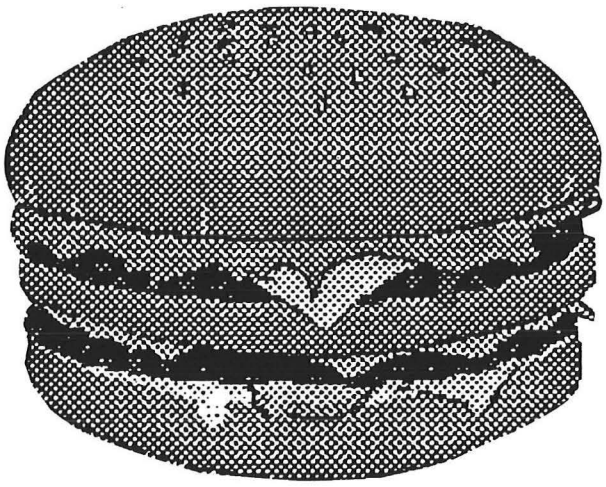
E. coli O157:H7 Infections



Internal Medicine Grand Rounds
University of Texas Southwestern Medical School

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November 20, 1997

This is to acknowledge that Byron Cryer has no financial disclosures, no financial interests or other relationships with commercial concerns related directly or indirectly to this program.

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INTRODUCTION

In the 15 years since the initial recognition of *Escherichia coli (E. coli)* O157:H7, this organism has emerged as a major pathogen for sporadic cases and outbreaks of diarrhea, both bloody and non bloody, and is known to be responsible for most cases of the hemolytic-uremic syndrome in North America. Its biochemistry and pathophysiology have led to the characterization of a new category of *E. coli*, the enterohemorrhagic *E. coli*, a pathogenic category that had not been recognized prior to identification of serotype O157:H7. In the United States alone, *E. coli* O157:H7 is estimated to cause more that 20,000 infections and as many as 250 deaths each year[1].

BACKGROUND

In February 1982, a gastroenterologist in Oregon evaluated, in rapid succession, three cases unlike any others he had seen before. The patients had what, at that time, was considered to be an unusual gastrointestinal illness characterized by severe bloody diarrhea, abdominal cramps with little or no fever. Stool cultures were negative for known pathogens. After this physician notified the state health department of these cases, a systematic review of medical records from local hospitals identified 25 other similar cases. A few months later another outbreak of a similar clinical illness was reported in Michigan[2]**(Figure)**.

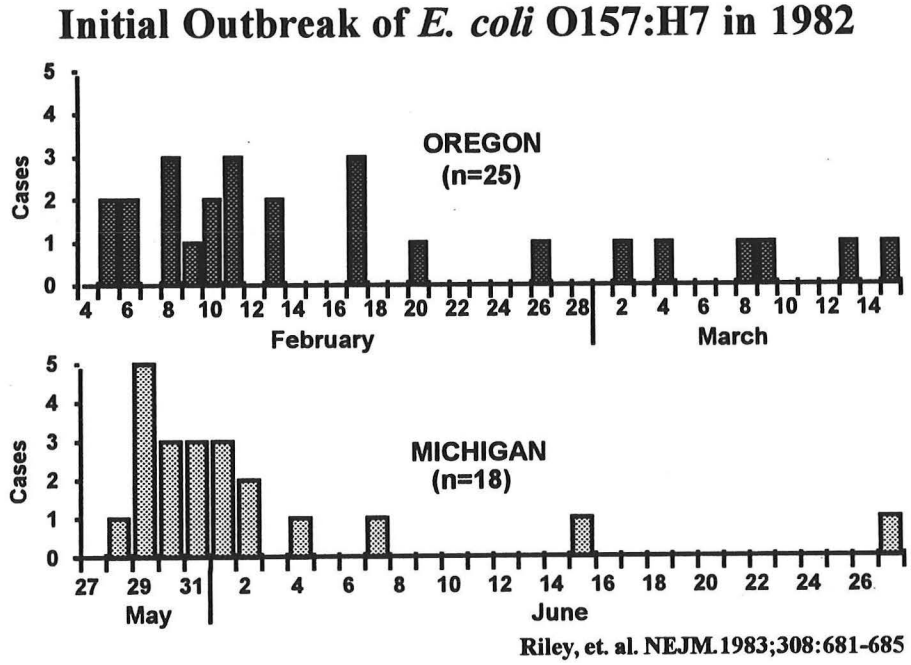


Figure. Cases of Hemorrhagic Colitis Described in Initial 1982 Outbreak[2]

Extensive epidemiologic and laboratory evaluations led to identification of *E. coli* O157:H7 as the pathogen which caused these bloody diarrheal illnesses and traced the vehicle for these infections to contaminated, undercooked ground beef from a chain of fast-food restaurants[2,3]. In the early 1970s,

ten years prior to identification of *E. coli* O157:H7 in 1982 as a pathogen in hemorrhagic colitis, this syndrome was recognized and reported under several names, including evanescent colitis[4], transient ischemic colitis, and transient hemorrhagic colitis[5]. In 10 years following the initial 1982 outbreaks in Oregon and Michigan, approximately 30 outbreaks were recorded in the United States[6]. *E. coli* O157:H7 has since emerged as an important cause of both hemorrhagic colitis and hemolytic uremic syndrome, and is the most common cause of acute renal failure in children. Outbreak investigations have linked most cases with the consumption of contaminated, undercooked ground beef products, although other food vehicles, including roast beef, raw milk and unpasteurized apple cider have also been implicated.

This organism, *E. coli* O157:H7, and its associated diseases, have provided excellent examples for study of a number of aspects of clinical medicine, specifically, epidemiology, microbiology, pathophysiology, cellular biology and preventive medicine. This review will cover each of these areas using *E. coli* O157:H7 as an illustrative example.

EPIDEMIOLOGY

Because few laboratories in the United States routinely culture stool specimens for *E. coli* O157:H7[7], the actual incidence of infection with the organism is unknown. However, the best data to suggest its incidence comes from a prospective, population-based study conducted in the Seattle area in 1985 to 1986 which reported an incidence of 8 infections per 100,000 persons per year[8]. When this estimate is applied to the United States population, *E. coli* O157:H7 is estimated to cause 21,000 infections in the U. S. annually [1].

In North American and European studies comparing the rates of isolation of *E. coli* O157:H7 and other common bacterial enteric pathogens in sporadic diarrheal illnesses, *E. coli* O157:H7 is not uncommon (~1% prevalence) and, in general, is isolated more commonly than *Shigella*, *Aeromonas* and *Yersinia* [8-12] **(Table)**. In sporadic cases of bloody diarrhea, the prevalence of *E. coli* O157:H7 as a pathogen dramatically jumps to up to 39% of cases [13-16] **(Table)**. Furthermore, in epidemic outbreaks of hemorrhagic colitis, *E. coli* O157:H7 is diagnosed in up to 60% of cases and is implicated in almost all cases.

Frequency of Isolation of Common Pathogens in Stools of Patients with Diarrhea

Non-Bloody Diarrhea

Pathogen	Percent
Campylobacter	2.7%
Salmonella	1.5%
E. coli O157:H7	0.9%
Aeromonas	0.8%
Yersinia	0.8%
Shigella	0.3%

Bloody Diarrhea

Pathogen	Percent
E. coli O157:H7	39%
Campylobacter	11%
Salmonella	8%
Shigella	2%

The number of outbreaks of *E. coli* O157:H7 infections reported to the Centers for Disease Control and Prevention (CDC) has dramatically increased in recent years from 4 outbreaks in 1992[1] to 64 outbreaks in 1994 and 1995[17]. In large part, this increase is attributable to increased reporting and screening by laboratories for *E. coli* O157:H7.

Modes of Transmission of *E. coli* O157:H7 Infections

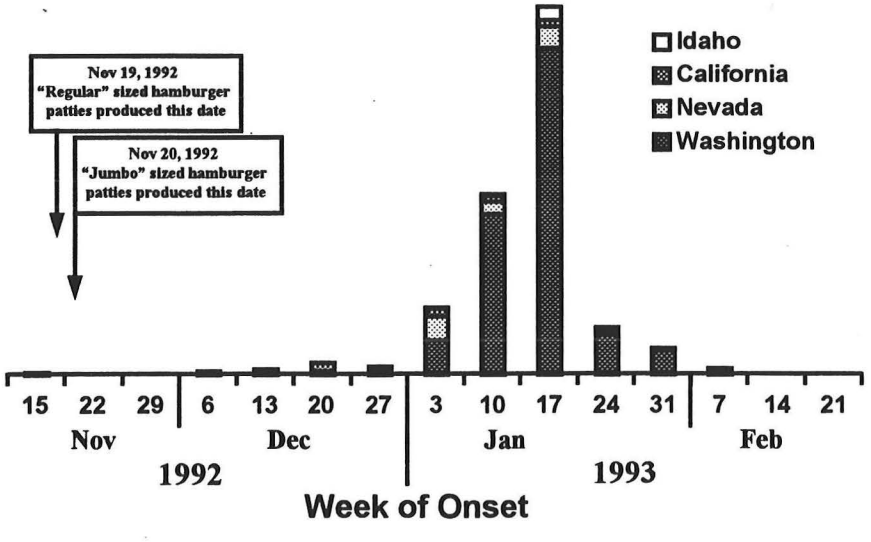
Most information regarding modes of transmission of *E. coli* O157:H7 infection has been derived from investigations of the numerous outbreaks that have now occurred in the 15 years since its initial recognition. Outbreaks have occurred in the United States[18-22], Canada [16,23,24], South America[25,26], Europe[27-34], Africa[35], Australia[36], India[37], and Japan[38-40], with cases in the U.S. being the most frequently reported. Careful investigations of these numerous outbreaks have helped define the clinical spectrum of illness and modes of transmission of *E. coli* O157:H7. As examples of the various modes of transmission, three case-control studies are summarized in the following sections, each selected because of its historical significance and impact on our understanding of *E. coli* O157:H7 infections.

Beef

In the United States, more outbreaks have been traced to contaminated ground beef than to any other vehicle.

1992-1993 Western U.S. Outbreak of *E. coli* O157:H7 from Hamburgers. Although, isolates of *E. coli* O157:H7 were first implicated in foodborne illness in 1982, notoriety within the lay community and an improved understanding of *E. coli* within the scientific community was achieved after a large outbreak occurring in 1992-1993 in the Western United States. Within a 14 week period, from November 15,1992 through February 28, 1993, more than 500 confirmed cases and four deaths from *E. coli* O157:H7 occurred in four Western U.S. states: Washington, Idaho, California and Nevada[20]**(Figure)**.

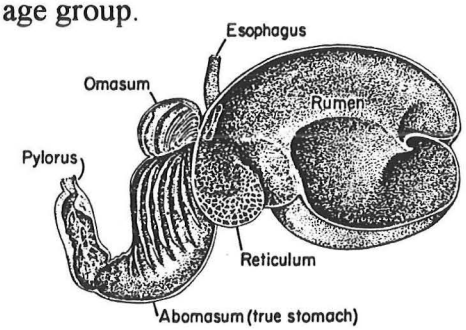
Cases of *E. coli* O157:H7 in 1992-1993 Western U.S. Outbreak



All associated illnesses in this multistate outbreak resulted from consumption of contaminated, undercooked hamburgers which had been purchased from one restaurant chain. All cases had clinical presentations of either bloody diarrhea or a post-diarrheal hemolytic uremic syndrome. A case-control study comparing case-patients who ate at a particular restaurant chain to meal companions with no clinical illness implicated regular sized hamburger patties, and in particular, contaminated frozen beef patties as being the source of the infection. A meat traceback by a CDC team identified five slaughter plants in the United States and one in Canada as the likely sources of carcasses used in the contaminated lots of meat. *E. coli* O157:H7 was isolated from 11 lots of patties produced on those two dates: Nov. 19, 1992 (regular patties) and Nov. 20, 1992 (jumbo patties)**(Figure)**[20].

Those 11 contaminated lots were distributed to the implicated restaurants in all four states where illnesses occurred. The animals slaughtered in domestic slaughter plants were subsequently traced to farms and auctions in six western states. Interestingly, not all confirmed cases had eaten in the implicated restaurant chain. During the week preceding onset of symptoms, 11% of infected individuals had been in close contact with a person confirmed to have an *E. coli* 0157:H7 infection. This observation of person-to-person transmission in this Western U.S. epidemic was of historical importance because it led to further investigations on the mode of transmission within families, child day care centers and in other institutional settings.

Reservoir & Vehicle for Hamburger-Related *E. coli* Infections. Microbiologic surveys indicate that up to 5% of clinically healthy cattle shed *E. coli* O157:H7 into their feces[41-46]. Younger cattle are apparently more susceptible to this infection and are more likely than adult cattle to shed *E. coli* O157:H7.After experimental inoculation, younger animals shed *E. coli* for longer periods of time[43,44]. Neonatal calves (< 3 weeks old) inoculated with *E. coli* O157:H7 develop a clinical diarrheal illness[47,48].On histologic evaluation, the organism adheres to their intestines and causes characteristic histologic lesions[47,48]. In contrast, in older (>3 week) calves and in adult cattle, clinical abnormalities have not been noted in those that are naturally infected with *E. coli* O157:H7 nor in those who have been experimentally inoculated with the organism[42-46]. In experimentally inoculated older animals, the organism does not adhere to or efface gastrointestinal epithelium, yet only localizes to certain areas of the gastrointestinal tract, namely the forestomach [45]. Thus, even though *E. coli* O157:H7 localizes within GI tract of slaughter cattle and can be fecally shed, it does not adhere to intestinal mucosa of older animals and is not a pathogen in this age group.

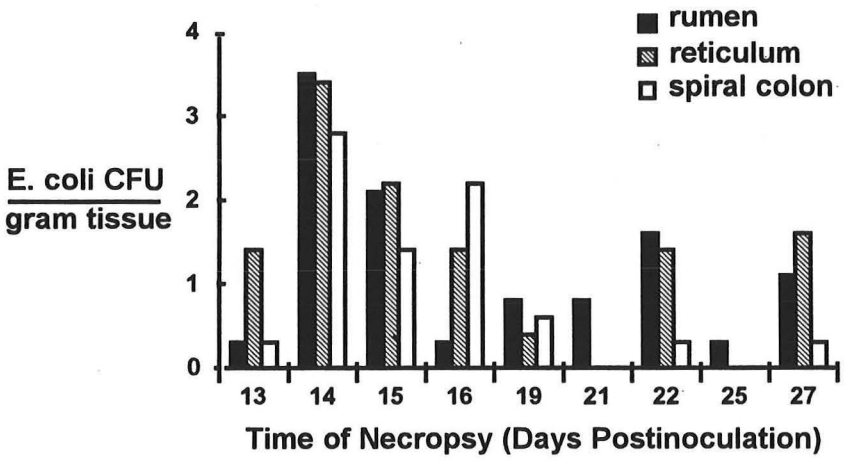


Ruminants, such as cow, sheep and deer, which are known vehicles for *E. coli* O157:H7 infections[41,49], have specialized four compartment stomachs[50] **(Figure)**. Their four compartment stomach is specialized for digestion of the complex fibers which are the principal component of their diets. Food, when eaten, descends into the first two of these compartments, the rumen and the reticulum. The former is a large pouch, the latter a smaller accessory chamber whose interior surface consists of a cris-cross series of ridges with deep intervening pits ("honeycomb tripe")[51]. In

these two chambers, volume is added and food is reduced to a pulp by a kneading action of very muscular walls while being simultaneously subjected to fermentation through the action of bacteria and protozoans which break down complex plant material (primarily cellulose). At this point the animal regurgitates the "cud" for further chewing (or rumination). On the second descent the cud bypasses the rumen and reticulum, and is routed via the omasum into the acid secreting portion of the stomach the abomasum (true stomach). The abomasum contains all three types of epithelium distinctive of mammalian stomachs, cardiac, fundic and pyloric gland mucosa[51].

Because acidic environments are not conducive to bacterial growth, most *E. coli* O157:H7 in cattle are found in the forestomach (rumen, reticulum, and omasum)[45]. No *E. coli* O157:H7 are found in the abomasum and very few are observed in the proximal intestine[45]. Since some strains of *E. coli* O157:H7 have a high level of acid tolerance[52], a portion of the bacteria originating in the forestomach will eventually localize in the distal small intestine and colon[45]**(Figure)**. In the colon, the organism is shed and can be cultured up to 4 weeks after experimental inoculation [45].

Recovery of *E. coli* from Infected GI Tissues



Appl.Environ.Microbiol.1997;63:27-32

Since *E. coli* O157:H7 is not known to infect extraintestinal sites, beef products which are not ground, steaks for example, are not felt to be at particularly high risk to be contaminated with the organism. In contrast, the high frequency of ground beef contamination is usually a consequence of processing. When beef is ground, infected intestines may be inadvertently added. Furthermore, because beef grinding during slaughtering may transfer pathogens from the surface of meat to the interior, ground beef is likely to be internally contaminated.

Non-Beef Modes of Transmission

In the last several years, several foodborne outbreaks of *E. coli* O157:H7 have implicated unique and seemingly unlikely vehicles of infection. Among them are acidic foods, fruits, salad vegetables, yogurt and water.

Acidic Foods

According to the U.S. Food and Drug Administrations's safety codes, food with a pH of less that 4.6 (acidic foods) are generally regarded as low risk in terms of food safety and potential for transmission of infection. However, several recent reports of disease outbreaks of *E. coli* O157:H7 have demonstrated that this pathogen can persist in acidic foods.

Apple Cider. In the fall of 1991, an outbreak of *E. coli* O157:H7 that affected 23 persons in southeastern Massachusetts occurred. Epidemiologists from the CDC used a case-control study to determine the vehicle of infection and traced it to a seemingly unlikely vehicle, fresh-pressed, non-pasteurized apple cider [53]. In the study design a "case" would be defined as one who had a diarrheal illness or hemolytic-uremic syndrome in the Fall River area of Massachusetts during the epidemic time frame and who had his *E. coli* O157:H7 infection confirmed by culture or serology. Eighteen cases were identified. A total of 49 age-, sex-, and neighborhood-matched controls were identified (2 to 3 controls for each case) who had not had an illness during this time frame. All study subjects were questioned regarding illness, food consumption, shopping practices and meat preferences.

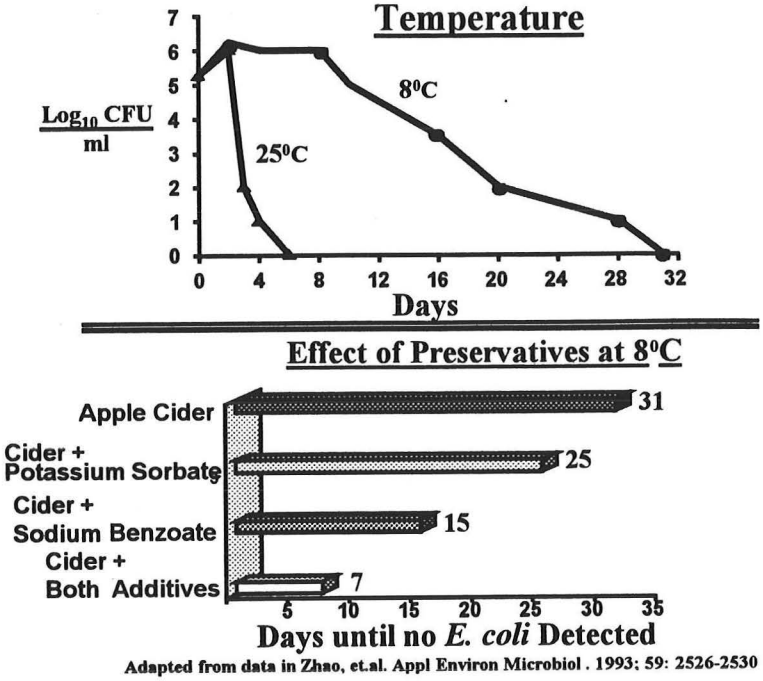
Frequency of Exposure to Selected Foods
Fall River Area, Massachusetts
October and November 1991

Exposures	No. of Patients/Total (%)	No. of Control/Total (%)	Matched Odds Ratio (95% Confidence Interval)
Apple cider	13/18 (72)	16/49 (33)	8.3 (1.8 to 39.7)
Cider from cider mill A, all patients and controls	12/18 (67)	2/49 (4)	34.0 (4.3 to 270.0)
Hamburger	7/18 (39)	39/49 (80)	0.2 (0.0 to 0.6)

Epidemiologists discerned that illness was significantly associated with drinking one brand of apple cider. 72% of patients but only 33% of 49 controls reported drinking apple cider in the week before illness (odds ratio [OR]=8.3). Moreover, consumption of a particular brand of apple cider from mill A was reported by 67% of patients but in only 4% of controls, OR=34.0. When the mill's processing practices were evaluated, it was determined this mill used unwashed "dropped" (picked from the ground) apples from their farm. It was further suggested, but never proven, that the dropped apples were contaminated with infected manure droppings from cattle in the field next to the apple orchard. These never-washed apples were pressed and their juice, which was not pasteurized, and contained no preservative, was distributed. Since this outbreak, there have been a number of other outbreaks associated with unpasteurized apple cider and apple juice[54-57].

It is of interest that the implicated apple cider was moderately acidic having a pH of 3.7 to 3.9, conditions which would be inhospitable to most enteric pathogens. It was subsequently demonstrated, however, that *E. coli* O157:H7 can tolerate acidic conditions. Some strains persist in media with pH values as low as 2.0 [58] and in cold (8°C) apple cider for 10 to 31 days [53,59].

In attempts to reduce the infectious risks associated with fruit juices, the CDC has evaluated the effects of various parameters on *E. coli* O157:H7's viability in apple cider. Using apple cider spiked with organisms from an outbreak, it was observed that while the bacteria will survive up to 31 days at 8°C, its survival is only 2 to 4 days at 25°C[59] **(Figure, top)**. When preservatives such as potassium sorbate or sodium benzoate are added alone or in combination to cider at 8°C, bacterial survival can be reduce from 31 days to 7 days[59]**(Figure, bottom)** Since neither of these conditions consistently or quickly kills organisms, the CDC recommends drinking only pasteurized or boiled apple cider or juice until alternative effective measures are developed[54].



Mayonnaise & Mayonnaise-based dressings. The ability of *E. coli* O157:H7 to tolerate acidity was substantiated in 1993, when mayonnaise (another acidic food), was implicated in a series of restaurant outbreaks that infected at least 48 persons. Samples of mayonnaise which were found to be infected with *E. coli* O157:H7 had a pH of 3.6 to 3.9, and the sauces prepared from it were also acidic, with pH levels for 3.6 to 4.4[60]. After this outbreak, several studies confirmed that although isolates of *E. coli* O157:H7 do not multiply under these conditions, they can persist in commercial mayonnaise up to 55 days at 5°C[60,61]. The manner in which the mayonnaise became contaminated with *E. coli* O157:H7 was never determined. However, improper handling of bulk mayonnaise or cross-contamination with meat juices or meat products was suspected.

Milk. Cow's milk has been documented as a vehicle of *E. coli* O157:H7 infection. Two cases of pediatric hemolytic uremic syndrome investigated by the CDC in 1986 were the first to be linked to raw milk consumption[62]. The milk in these cases came from two separate farms, both of which had heifers test positive for *E. coli* O157:H7. One of 23 raw milk specimens collected from the farms at a later date also tested positive[63]. Outbreaks have also occurred in Canada[64] and the United Kingdom[65,66] where infection was significantly associated with drinking unpasteurized milk[67]. Of greater concern, pasteurized milk[68] and yogurt[69-71] have also been implicated in outbreaks. In one of these instances, genotypically similar *E. coli* O157:H7 isolates were recovered from contaminated milk samples and the bottling machinery[68].

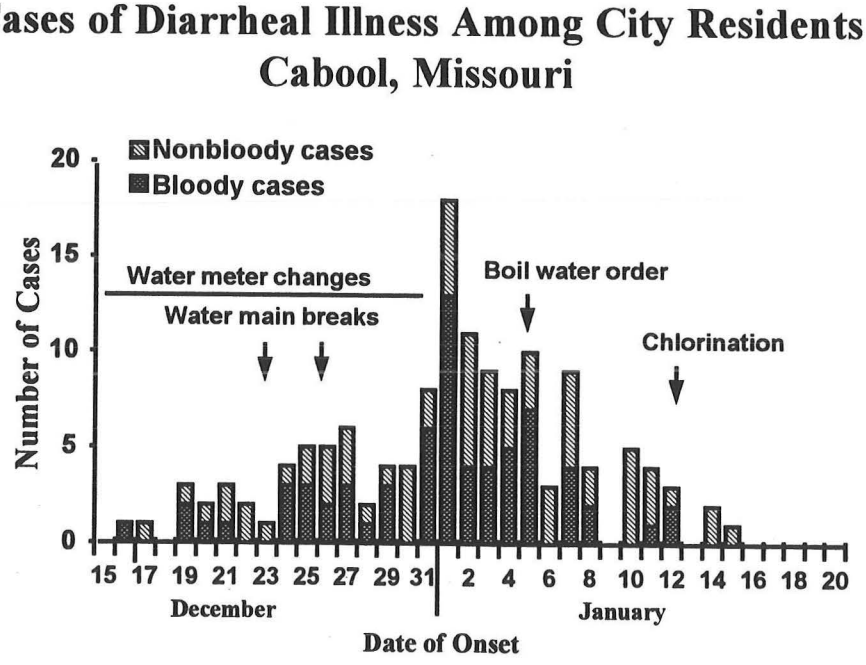
Other Food Vehicles of Transmission. Recently, several other unique vehicles have been implicated in foodborne outbreaks associated with *E. coli* O157:H7. A 1993 outbreak in an Oregon restaurant was apparently caused by the consumption of cantaloupe or other items from the salad bar. It was thought that many salad bar items were most likely cross-contaminated by meat products during preparation. In one of the studies of salad bar-associated outbreaks, it was demonstrated that *E. coli* O157:H7 can survive and grow on salad vegetables stored either at 12°C or 21°C for up to 14 days [72].

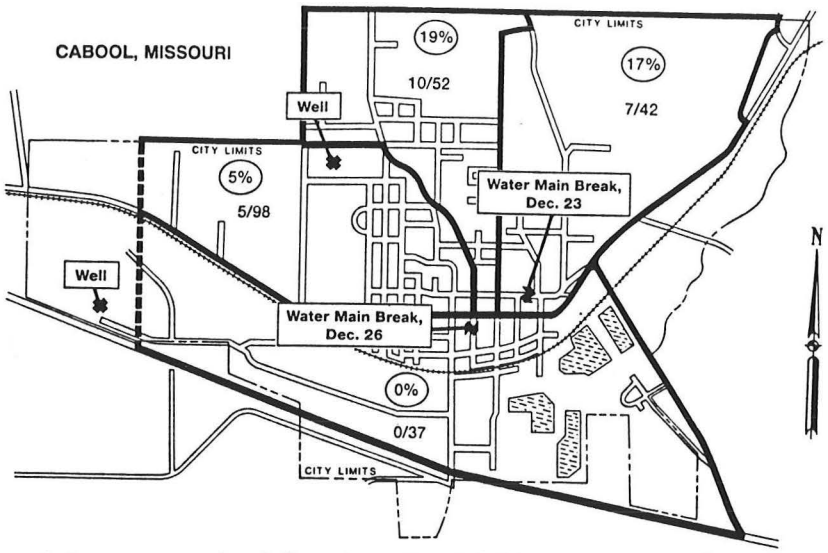
Chicken has also been implicated as a possible vehicle for transmission of *E. coli* O157:H7[34,73-75]. In an epidemic which occurred in northern Italy where 15 cases of the hemolytic-uremic syndrome were reported, epidemiologic data suggested that contact with live poultry or with chicken coops may have been the source of the infection, even though no pathogenic *E. coli* strains were isolated from poultry feces[34]. A recent study has shown that inoculating 1 day old chicks with strains of *E. coli* O157:H7 results in rapid colonization of the chick's cecal tissue[75]. The chicks will maintain their infections up to 11 months, constantly shedding the organism into their feces. Furthermore, *E. coli* O157:H7 can be recovered from the shells of these chicken's eggs[75].

Dry cured salami[74], sausage[76], venison jerky [49], and alfalfa sprouts[77] have all additionally been implicated as vehicles for foodborne *E. coli* infections. Although consumption of infected bovine products continues to account for most *E. coli* O157:H7 infections, the numerous previously described cases demonstrate that, not uncommonly, other food types can also serve as vehicles for transmission of this infection.

Water. Several recent incidents have shown that both drinking water[78-80] and recreational water[81-85] can serve as vehicles for transmitting *E. coli* O157:H7 infections.

Cabool, Missouri. The first and largest waterborne outbreak occurred in Missouri in 1989[78]. In Cabool, a small city in rural southern Missouri, a large outbreak of *E. coli* O157:H7 infection was traced to drinking water from a municipal water system. Between December 12 and 24 1989, the region experienced extreme cold, with low temperatures ranging from -28°C(-19°F) to 0°C (32°F). During this cold period 45 water meters connected to the municiapl water system were changed and, on Decmber 23 and December 26, two large water mains broke.





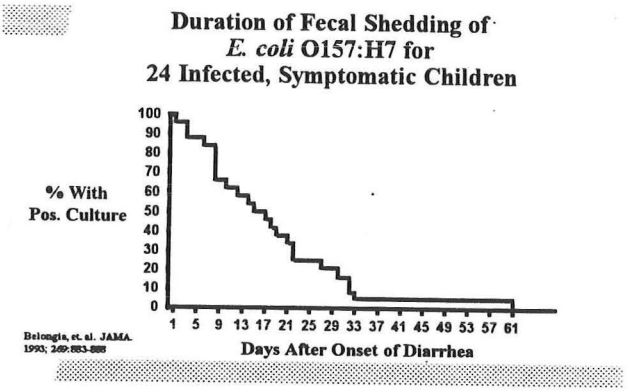
In this time frame, 240 people were identified who were infected with *E. coli* O157:H7, 32 were hospitalized and four died. These patients' stool specimens yielded no pathogens using routine culture methods, however, *E. coli* O157:H7 was isolated for their stools when special culture medium was used. Epidemilogists from the CDC performed a case-control stduy of households with the Cabool township and the U.S. environmental proctetion agency (EPA) performed environmental evaluations. The source of this outbreak was not identified. However, it was speculated that sewage backflow into the drinking water supply during the water main breaks might have been the source of *E.coli* contamination. Of particular interest, when the EPA applied the city's water-sytem flow data to a computer model, it was demonstrated that bacteria introduced near the sites of water main breaks would cause the highest concentrations of *E. coli* O157:H7 infection in the north and norteastern parts of the town (the sections of town which indeed had the highest rates of infection **(Figure)**. The other interesting observation dervied from the Cabool experience was that cases of diarrheal illnesses declined after an residents began to boil water and chlorine was added to the water supply **(Figure)**. After the water mains were repaired in Cabool, no disinfectant was added to the water system. Like most *E. coli*, serotype O157:H7 are susceptible to chlorine. Therfore, the observations derived from this case control study in 1992 led to an EPA proposal to enact legislation which would require addition of disinfectants such as chlorine to all water supplies and use of hyperchlroination during repairs of water system breaks.

Recreational Water Outbreaks. Transmission of infection in recreation lake water has been suggested as the source of a number outbreaks of *E. coli* O157:H7-associated illnesses[81-84]. An epidemiologic survey of one outbreak in Portland, Oregon in 1991 demonstrated that all who became ill had swum in the lake during the previous 3 week period[81]. Transmission probably occured when the swimmers swallowed lake water that was fecally contaminated by other bathers. The lengthy period during which people became infected suggest that these pathogens can remain viable in water for a long time or that the water was repeatedly contaminated. When fecal contamination of recreational water occurs, the contaminants are usually diluted quickly by the large volume of water. The observation that swallowing a small amont of lake water can cause disease suggests that the pathogen has a low infectious dose[81], an obeservation that is consistent with data from the foodborne outbreaks associated with *E. coli* O157:H7.

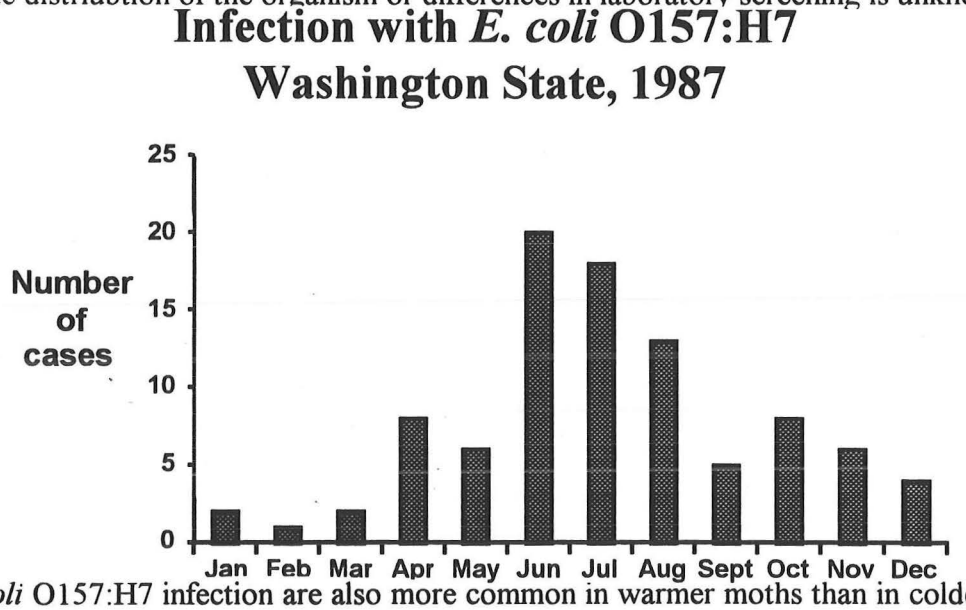
Swimming Pools. Similar to the recreational water outbreaks, cases implicating contaminated water from swimming pools have been reported[85]. Transmission in these cases are also thought to be assoicated with fecal contamination of pool water which has no disinfectant.

Person-to-Person Transmission

Improper hygiene with secondary spread from person-to-person contact is another well-documented route of infection[86] which is in large part attributable to the low infectious dose required for disease production. Outbreaks have been docemented in nursing homes[87,88], schools [89] and day-care centers[86,90,91], and mental institutions . [92]Cases of sporadic transmission to hospital workers have also been described[93,94]. From an infection control point of view, it is of interest that the organism may be shed into the stool for weeks after the diarrheal illness has resolved and patients have become asymptomatic[86,91,95]. As shown **(Figure),** duration of fecal shedding in children with *E. coli* O157:H7 infections in day care was determined from the date of onset of diarrheal illness. In most cases of *E. coli* O157:H7 infections, the diarrheal illness lasts about 7 days. It is of interest that at 17 days after onset of symptoms, 50% of the affected children continue to shed organisms in their stool and one child shed *E. coli* for 61 days, long after the diarrhea resolved[86].



Geographic and Seasonal Factors. In the United States, sporadic cases and outbreaks are more frequently reported in the northern states than in the southern states. Whether this variation reflects a true distriubtion of the organism or differences in laboratory screening is unknown.

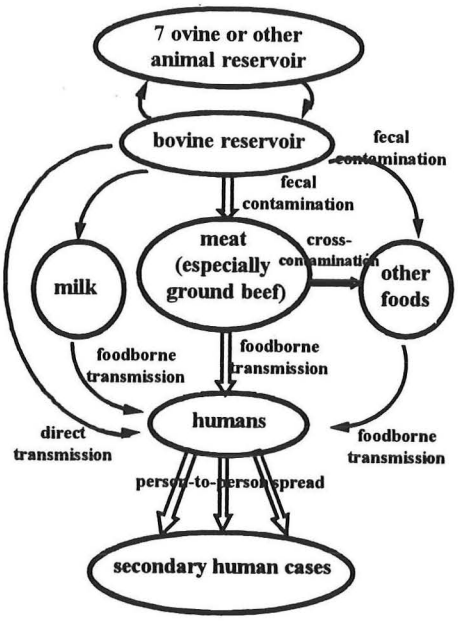


E. coli O157:H7 infection are also more common in warmer moths than in colder months, having a peak incidence in June though September[11,21] **(Figure)**. The reason for this seasonal variation is unknown. It may reflect the ecology of the organism, increased amounts of *E. coli* in the food supply during the summer months, factors related to its reservoir in cattle or related to farming practice, variations in ground beef consumption or cooking practice, or to some other factor[1].

Outbreaks of *E. coli* O157:H7 Reported to the Centers for Disease Control and Prevention (CDC) from 1982 to 1994 Inclusive

Likely vehicle or mode of spread	No. of outbreaks	No.of individuals involved
All foods	38	1,541
Ground beef	22	1,137
All food products and milk	26	1,278
Drinking water or swimming-associated	3	276
Person-to-Person (no food identified)	9	243
Unknown	19	274
All outbreaks	69	2,334

Reservoirs and Modes of Transmission of *E. coli* O157:H7



ORGANISM

Antigenic Structure

Antigens present on the surface of *E. coli* O157:H7 have been traditionally used to classify *E. coli* serotypes. The three major classes of antigens are: 1) the O antigens or somatic antigens, 2) the H or flagellar antigens, and 3) the K or capsular antigens **(Figure)**.These major anitgenic groups are present and conserved in all Enterobacteriaceae species[96].

The O antigens consist of the polysaccharide side chains of the LPS envelope found in all gram-negative bacteria. In *E. coli* species in general, epidemiologic data have suggested that the O serogroup may primarily act as a marker for a specific clustering of virulence properties that are required for organisms to sucessfully manifest an infection[96]. The H antigens are proteins which are found on bacterial flagella, the structural aspect which allows many gram-negative bacteria to be motile. The K antigen is typically located on the polysaccharide capsule.

Classification of *E. coli*

At least six different classes of diarrhea-producing *E. coli* have been identified[96-100] **(Table)**. Diarrhea caused by **enterotoxigenic** *E. coli* (ETEC) is generally a self-limited illness of varying severity which presents as watery stools and abdominal cramps. ETEC infection occurs in persons of all ages, and is an important cause of travelers' diarrhea, diarrhea in infants in developing countries, and "food poisoning" syndromes associated with upper gastrointestinal symptoms such as nausea and vomiting. **Enteroinvasive** *E. coli* (EIEC) cause dysentery, similar to that caused by *Shigella*, and is characterized by fever, diarrhea, vomiting, crampy abdominal pain and tenesmus. Stool often contain blood and white blood cells. Diarrhea caused by **enteropathogenic** *E. coli* (EPEC) typically occurs in neonates and children less that 1 year of age in developing countries or in association with outbreaks in newborn nurseries. Illness is characterized by acute, watery diarrhea that is often severe and many result in dehydration or chronic diarrhea that can cause failure to thrive.

Types of *E. coli* Intestinal Pathogens

Strain	Clinical Features	Pathogenic Mechanism
Enterotoxigenic (ETEC)	**Traveler's diarrhea**	**Elaboration of secretory toxins: LTs & STs; Non-invasive No damage to host epithelium**
Enteroinvasive (EIEC)	**Dysentery (Fecal WBC's & RBC's)**	**Invade cells / Destruct intestinal mucosa Significant inflammatory response**
Enteropathogenic (EPEC)	**Nonbloody watery Children<2 Newborn nurseries**	**Epithelial adherence in microcolonies Attaching & Effacing (A/E) lesions**
Enterohemorrhagic (EHEC)	**Hemorrhagic colitis**	**No enteroinvasion Elaboration of Shiga-like Toxin**
Enteroaggregative (EAgg EC)	**Acute and chronic diarrhea in children in developing countries**	**Aggregative adherence to epithelial cells**
Diffusely Adhering (DAEC)	**Acute and chronic diarrhea in children in developing countries**	**Diffuse adherence to epithelial cells**

Prior to the identification in 1982 of *E. coli* O157:H7 as a pathogen, the above three classes of *E. coli* were the three best characterized pathogenic classes to date. *E. coli* O157:H7 led to characterization of an additional class of *E. coli, the* **enterohemorrhagic** *E. coli* (EHEC) strains of which *E. coli* O157:H7 is the prototype. All EHEC strains have the same clinical, epidemiologic and pathogenic features as *E. coli* O157:H7. **Enteroaggregative** *E. coli* (EaggEC) and **diffusely adhering** (DAEC) infections are associated with acute and chronic watery diarrhea in children in developing countries.

In addition to characterization of the basis of their clinical effects, the various diarrheagenic *E. coli* strains can be segregated by distinctive pathogenic mechanisms. ETECs are non-invasive, produce secretory [heat labile (LT) and heat stable (ST)] toxins that don not damage the host's mucosal epithelium. EIEC penetrate epithelial cells and destruct intestinal mucosa. EPEC adhere to epithelial cells in localized microcolonies and cause the attaching and effacing (A/E) lesions in animals. EaggEC adhere to cultured epithelial cells in an aggregative or "stacked brick" pattern while DAEC adhere to these cells in a diffuse pattern.

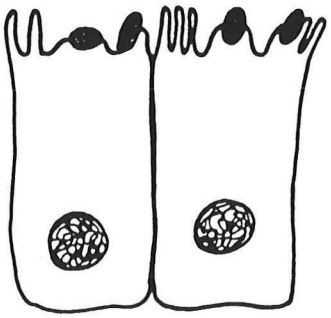
This classification system, while providing a frame of reference, oversimplifies the situation because it treats categories as mutually exclusive combinations of virulence traits, which they are not. For example, ETEC and EPEC can invade cells in culture[79,80]. Furthermore, as discussed below EHEC can cause the A/E lesions (at least in animal models) that are characteristic of EPEC. Therefore, since it is apparent that there is overlap in pathogenic mechanisms of diarrheagenic strains of *E. coli,* strict classification of these organisms may no longer be possible.

Virulence and Virulence Factors of Enterohemorrhagic *E. coli*

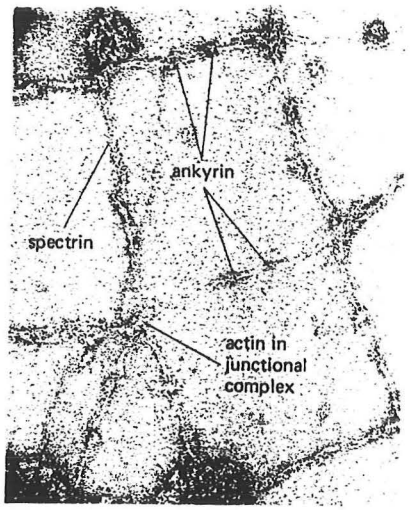
There are two primary features which determine virulence among *E. coli* O157:H7 species: adherence to intestinal epithelium and toxin production.

I. **Colonization and Adherence:** The attachment of an organism to an intestinal epithelial surface is often a prerequisite for its pathologic effect. The preferred intestinal sites for attachment of the EHEC are terminal ileum, cecum and proximal colon. The initial step is association of fimbriae with intestinal epithelial cells[101]. These surface organelles that allow adhesion are termed fimbriae or pili, hair-like organelles that radiate from the surface of the cell in all directions. This process is encoded by plasmids which carry adherence factor sequences. Following fimbriae-associated attachment, there is a more intimate attachment which is encoded for by chromosomally encoded gene products[102-104] and described below.

When EHEC intimately adhere to intestinal tissue of animal models, a characteristic histopathological lesion is produced. This lesions is called the attaching and effacing lesion (A/E) and is characterized by dissolution of the brush border membrane and loss of microvillus structures (effacement) at sites of bacterial attachment[102,105-107]**(Figure).** The organisms adhere intimately to cup-like projections or pedestals of the apical membrane. In the intestinal epithelial cell, high concentrations of filamentous actin derived from cytoskeleton are present beneath the site of bacterial attachment. ETEC, EIEC, and nonpathogenic *E. coli* do not produce this lesions[105]. Among *E. coli,* the A/E lesion appear to be a specific marker for EPEC and EHEC.



In response to bacterial attachment, there is transient elevation in intracellular calcium levels which leads to a break down of the cytoskeletal actin which supports the microvillus core and ultimately results in villi flattening (effacement). Breakdown of cytoskeletal actin results in actin polymerization and actin aggregates. By incubating cells with fluorescent probes which bind to actin, intense spots of actin fluorescence are visualized **(Figure)**[108]. The fluorescent actin staining (FAS) reaction is an *in vitro* test which is employed



to determine the ability of *E. coli* to induce actin aggregation in cultured epithelial cells[102,105]. The FAS reaction is highly sensitive and a positive reaction is thought to correlate with an ability to produce A/E lesions *in vivo*[102,105]. Therefore, this test has been suggested as a non-invasive means to assess A/E lesion formation, thus obviating the need for electron microscopy of biopsied intestinal tissue[102,105].

II. **Toxins:** In the laboratory investigations of the initial 1982 outbreak of hemorrhagic colitis, it was discovered that *E. coli* O157:H7 had a pathophysiologic mechanism which was, at that time, unknown and was distinct from other known diarrheagenic *E. coli*. Outbreak-associated *E. coli* O157:H7 strains did not produce either heat-labile or heat-stable enterotoxin, nor were they enteroinvasive[3]. This new class of *E. coli*, O157:H7, produce either or both of two phage-encoded toxins which are genetically and biolgically similar to the toxin produced by *Shigellae dysenteriae*, shiga-toxin. Because of the similarities between the *E. coli*- and *Shigella*- elaborated toxins, the toxin produced by *E. coli* O157:H7 is called Shiga-like toxin or SLT. There are only three nucleotide and one amino acid differences between SLT and shiga toxin[109,110]. With respect to functional similarities, both *E. coli* O157:H7 and shigella toxins are: 1) cytoxic for the same cell culture lines (Vero and HeLa cells), 2) cause paralysis and death in mice, 3) caused fluid accumulation in rabbit ileal segments.[111,112] Furthermore, the toxin from some strains of *E. coli* O157:H7 are inhibited by an antibody to shiga-toxin. The observation that there were other *E. coli* O157:H7 strains whose SLTs could *not* be neutralized by antibodies to shiga-toxin suggested that *E. coli* O157:H7 produces two types of toxins[112]. Further genetic characterization of DNA encoding for these toxins led to the conclusion that *E. coli* O157:H7 produces two genetically homologous (58% homology[109]), but antigenically distinct cytotoxins which possess similar biologic activities[112].These toxins have been interchangably designated as verocytotoxins [or vertoxins (becuase of their toxic effects on cultured Vero cells)] or as Shiga-like toxins [SLT-I and SLT-II (because of their homology to the cytoxin produced by *Shigella dysenteriae* type1)][113,114]
(Table,top). Shiga toxins and SLTs are potent cytotoxic agents because they bind with high affinity to selective target cell types, enter those cells efficiently and inhibit cytoplasmic protein synthesis.

EHEC are class of *E. coli* that produce SLTs and refers to *E. coli* strains that have the same clinical, epidemilogic and pathogenic features as the prototype, *E. coli* O157:H7 **(Table, bottom)** Over 100 serotypes *E. coli* that produce SLT have been isolated from humans[101,115]. However, not all of these serotypes have been shown to cause disease. *E. coli* O157:H7 is the most common serotype of the EHECs which has been associated with human hemorrhagic colitis[101].

Toxin Nomenclature

Verocytotoxin
Verotoxin (VT)
Shiga-like Toxin (SLT)
Shiga Toxin

Bacterial Nomenclature

VTEC = Verotoxin-producing *E. coli*
SLT-EC = Shiga-Like Toxin-producing *E. coli*
EHEC = Enterohemorrhagic *E. coli*

In contrast to the Shiga-toxin gene of *Shigella*, the *E. coli* genes encoding Shiga toxins 1 and 2 are initially located not on the bacterial chromosome but on bacteriophages that infect *E. coli*. These bacteriophages may infect several different serotypes of *E. coli*, which in turn, incorporate the SLT message into their genomes and, thereby, acquire the ability to secrete shiga toxin. Thus, shiga toxin production and clinical disease have been reported with *E. coli* serotypes other than O157:H7. The **table** lists *E. coli* serotypes associated with hemorrhagic colitis and hemolytic uremic syndrome[116].

III. Other Virulence Properties. In addition to adhesins and toxins, the Enterobacteriaceae possess a number of additional auxiliary virulence factors. Flagella permit organism motility, scavenger molecules and receptors such as the siderophore system permit acquisition of limiting nutrients, and capsules block opsonophagocytosis and thereby foil the immunologic machinery of the host[96].

E. coli Serotypes Associated With Hemorrhagic Colitis and Hemolytic-Uremic Syndrome

O2:H5
O5:H-
O6:H-
O26:H11
O38:H21
O91:H-
O103:H2
O11:H8
O111:H-
O113:H21
O119:H6
O121:H19
O145:H-
O157:H7
O157:H-
O163:H19

CLINICAL MANIFESTATIONS

EHEC infection can cause a spectrum of illnesses which range from asymptomatic infection to death **(Table)**. *E. coli* O157:H7 is the only organism in this class for which extensive epidemiologic and clinical data are available. Although most of our knowledge regarding *E. coli* O157:H7 infections has come from outbreaks, it should be remembered that EHEC infections can occur sporadically or in outbreaks.

Spectrum of Human Conditions Caused by *E. coli* O157:H7

Condition	Frequency
Asymptomatic carriage	Unknown
Nonbloody diarrhea	≈ 10% of diagnosed cases
Hemorrhagic colitis	≈ 90% of diagnosed cases
Hemolytic-uremic syndrome	≈ 10% of infected patients < 10 yr old
Associated intestinal and extraintestinal complications	Fewer than 5% of cases

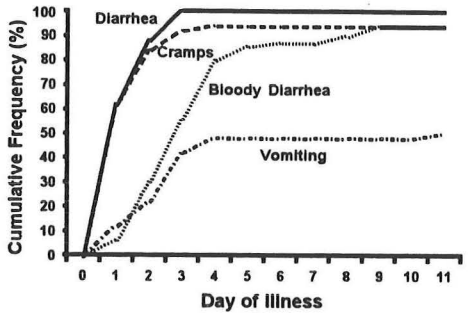
The prevalence of asymptomatic *E. coli* O157:H7 in humans is uncertain. It has been found, at least transiently in stool samples during screening of unaffected individuals in areas of outbreaks [99]. However, few statistics are available. An asymptomatic, long-term carrier state has not been identified.

Diarrheal Illness

After acquisition of an *E. coli* O157:H7 infection, there is a predictable typical progression of symptoms [117]. Illness typically begins with a prodrome consisting of severe crampy abdominal pain. Within one to two days, a watery, nonbloody diarrhea ensues which often becomes bloody by the second or third day of illness. However, about 10% of cases will experience non bloody diarrhea without progression to hemorrhagic colitis. Patients whose only manifestation of infection is nonbloody diarrhea have less severe disease that is less likely to progress to the hemolytic-uremic syndrome than is hemorrhagic colitis **(Table)**.

Stools of patients with *E. coli* O157:H7 are sometimes described as "all blood and no stool[2]. Alternatively, the diarrhea may be minimally blood-streaked or remain nonbloody. In reported outbreaks, the percentage of patients with *E. coli* O157:H7 who develop bloody diarrhea has varied widely, from 35% to 90%[78,118]. Thus, although bloody stools are common with *E. coli* O157:H7 infection, the diagnosis must be considered in patients with nonbloody diarrhea as well.

Sequence of Clinical Manifestations of Enterohemorrhagic *E. coli*



About one-third of patients have vomiting which may occur at any point during the illness during the illness. However, unlike most bacterial diarheal illneses, fever is usually low-grade or is absent[119].
Physical examination usually is unremarkable except for mild to moderate abdominal tenderness in about two-thirds of patients. In the absence of HUS, most laboratory tests are normal except for a mild leukocytosis. Small numbers of fecal leukocytes are detected in stool samples, but at a lesser degree than that seen with dysentric illnesses caused by invasive bacteria.

The lack of fever along with impressive abdominal pain and tenderness, sometimes leads clinicians to suspect noninfectious etiologies such as appendicitis, imflammatory bowel disease, ischemic colities, or in children intussusception **(Table)**. In many cases, concern about a possible surgical diagnosis sometimes leads to exploratory laparotomy[117,119].

Differential diagnosis of colitis caused by* E. coli *O157:H7.

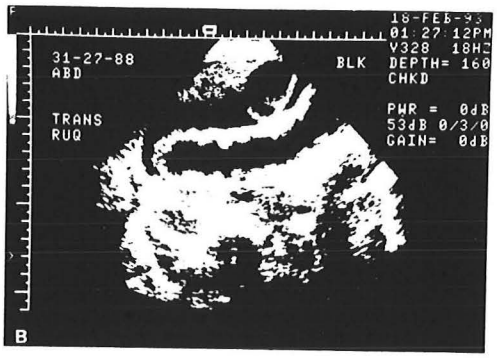
Infectious causes
Campylobacter
Clostridium difficile
Entamoeba histolytica
Salmonella
Shigella
Yersinia enterocolitica
Noninfectious causes
Appendicitis
Crohn's disease
Diverticulosis
Intussusception
Ischemic colitis
Ulcerative colitis

The incubation period is one to nine days during outbreaks and may be somewhat longer when it occurs in nursing homes[120].The diarrheal illness usually lasts between 4 to 10 days, although its length

is longer in children than in adults. On the worst day of illness, patients report a median of 10 to 11 bowel movements. In most patients, symptoms usally resolve without any serious complications obvious sequelae.

Radiographic Manifestations

X-rays may show ischemic changes suggestive of ischemic colitis, infectious colitis or inflammatory bowel disease[5,101,121]. Specifically, plain films of the abdomen will demonstrate an ileus in most patients. Plain films and/or barium enemas often reveal "thumbprinting" which is suggestive of submucosal edema and/or hemorrhage primarily in the ascending and transverse colon. The mucosa may have a shaggy appearance with thickened folds. CT may show marked intestinal wall thickening with associated mesenteric inflammatory changes[121]. Abdominal sonography may also demonstrate bowel wall edema with "thumbprinting"[101]**(Figure)**.



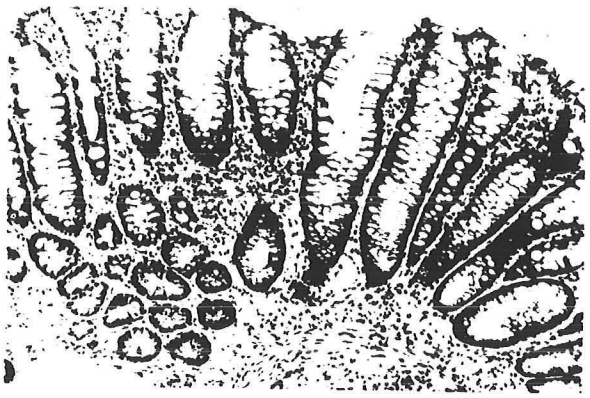
Endoscopic Manifestations

Endoscopic assessment of the colon, either by sigmoidoscopy or colonoscopy, is commonly employed in the evaluation of patients who present with bloody diarrheal illnesses. Such evaluations have allowed endoscopic and histologic characterization of colonic manifestations of *E. coli* O157:H7 infection. Endoscopic presentatons in hemorrhagic colititis include edema, erythema, erosions, superifical ulcerations or hemorrhage all of which are typically patchy in ditribution[122]. Also, the mucosa may have a dusky apearance or may be grossly bleeding. Pseudomembranes, although uncommonly reported, may also be present. In *E. coli* O157:H7 infections, the right-side of the colon is more commonly and more severely affected than the rectum or sigmoid colon[2,87,119,122,123]. The left colon may only show edema, erythema or may be spared entirely. In one colonscopic evaluation of patients with *E. coli* O157:H7 infections, only 45% of patients had involvement of sigmoid colon[122]. Therefore, sigmoidoscopy alone in patients presenting with hemorrhagic colitis has the potential to result in falsely negative evaluations.

Histology

Histologically there are no pathonomocic findings in biopsies of patients with *E. coli* O157:H7 infections. The most common observations are hemorrage and edema in the lamina propria. Another characteristic feature of *E. coli* O157:H7-associated colitis is that it resembles a combination of ishemic and infectious injuries similar to those described in *Clostridium difficile*-associated colitis,

and other toxin-mediated colonic infections[122]. The histologic pattern which is typical of the infections patern of injury demonstrates areas of focal infiltration of the lamina propria by focal inflammatory infiltrates which is sometimes associated with an inflammatory pseudomembrane **(Figure, top)**.



The ischemic type of injury shows superficial coagulative necrosis and mucosal hemorrhage which spares the deep crypt epithelium and can also be associated with an overlying inflammaroty pseudomembrane **(Figure, center)**.

At high power magnifications, small fibrin-platelet thrombi in mucosal capillaries can also be seen **(Figure, bottom)** which provides histologic corroboration that the pathophysioloy of *E. coli* O157:H7 injury in the colon is ischemic.

Hemolytic-Uremic Syndrome & Thrombotic Thrombocytopenic Purpura

Hemolytic uremic syndrome (HUS) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. Thrombotic thromocytopenic purpura (TTP), is characterized by the microangiopathic hemolytic anemia, thrombocytopenia, and renal failure seen in HUS, as well as by fever and central nervous system manifestations which can include confusion, seizures & coma.

Thrombotic Microangiopathies

HUS	*TTP*
Thrombocytopenia	Thrombocytopenia
Renal failure	Renal failure
Hemolytic anemia	Hemolytic anemia
Renal thrombi	Diffuse thrombi (heart, pancreas, adrenals, brain, kidneys)
	Neurologic changes
	Fever

HUS = Hemolytic Uremic Syndrome
TTP = Thrombotic Thrombocytopenic Purpura

At times, TTP is only partially clincially expressed as many patients do not present with the full clinical syndrome[124]. In contrast, although central nervous sytem complications are not part of the classic triad of conditions associated with HUS, they may occur in 30% to 50% of patients[125]. Because of overlap in clinical presentation, TTP and HUS are considered to

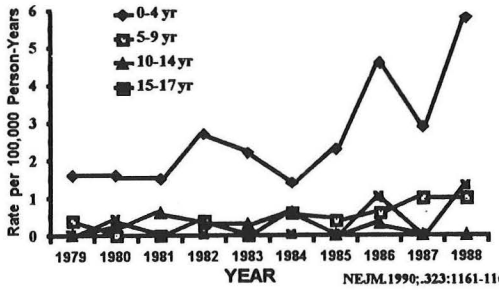
represent variations in expression of the same underlying disease [126,127]. Some, therefore, have proposed "thrombotic microangiopathies", as a preferred descriptive term which reflects their underlying pathophysiolgy and allows for variablity in symptom expression [124].

Based on epidemiologic, clinical and laboratory data, HUS can be divided into two groups, one associated with a prodromal diarrheal illness (epidemic form of HUS) and a second category in which no prodromal diarrheal illness is observed (sporadic form of HUS)[101,128]. Nondiarrheal HUS has numerous etiologic associations which include eclampsia, oral contraceptive use, malignant hypertension, collagen vascular diseases, chemotherapy, bone marrow transplantation, cyclosporine, HIV, familial and idiopathic[101,126,127,129].

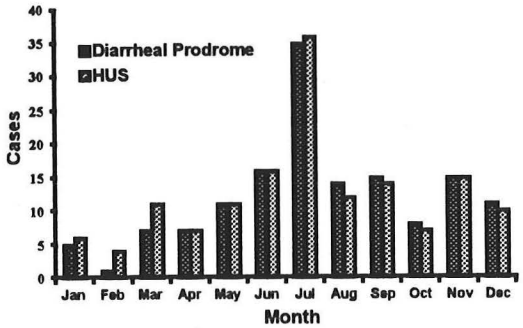
The HUS associated with prodromal diarrheal illness is the most common form. It is seen predominantly in infants and young children, is the most common cause of acute renal failure in young children[33,113,125,130,131], and has a mortality rate of 5 to 10% in this group[113,115]. The observations that epidemic cases of HUS occur after a prodromal diarrheal illness and that they tend to occur in clusters invovling families[132], day care centers[86], schools and nursing homes[87,88], led to studies evaluating the possiblity of an infectious etiology[111]. Infections which have been reported in association wtih HUS include: *Shigella dysenteriae* type 1, neuramidase-producing strains of pneumococci and a variety of other bacteria and viruses[101,133-136]. The common characteristic of all of the various agents suggested as causes for HUS is an ability to injure endothelial cells[137]. Shiga-like toxin (SLT), neuramidase, lipolysaccharides (LPS), phopholipase C, mitomycin-C, cylcosporin A can all damage endothelial cells[137].

Over the past 15 years since *E. coli* O157:H7 has emerged as an important pathogen, the incidence of HUS has been increasing[138,139] **(Figure, left)**. As seen in **Figure (left)** HUS primarily affects infants and small children. While HUS is predominantly a disease of young children, *E. coli* O157:H7-associated HUS has been observed in older children [130], young adults[140,141], the elderly [87] and in epidemic form in nursing homes [88].

Annual Age-Specific Incidence Rates of Hemolytic Uremic Syndrome in Minnesota, 1979-1988



Cases of Hemolytic Uremic Syndrome and Diarrheal Illnesses by Month in Utah in 1987



A cause and effect relationship between *E. coli* O157:H7 infection and HUS is further suggested by similar seasonal patterns of disease presentation[130]**(Figure, right)**. In *E. coli* O157:H7 outbreaks, where HUS symptoms have primarily occurred in the absence of a diarrheal illness, HUS has provided the diagnostic clue for the recognition of *E. coli* O157:H7 infection.

E. coli O157:H7 has also been associated with some cases of the sporadic form of HUS[111]. Whether sporadic or epidemic, about 75% of cases of hemorrhagic colitis complicated by HUS are caused by the O157:H7 serotype of *E. coli.* Infection with *E. coli* O157:H7 is now the factor most clearly linked to HUS, being found in 46% to 64% of patients[10,138,142]. Other EHEC serotypes, however, have been associated with hemorrhagic colitis and HUS[108], especially in South America[25,26] and Canada[113].

E. coli O157:H7 has also been implicated in several cases of TTP [143-145], which is thought to represent the severe end of the spectrum of clinical presentations of the .thrombotic microangiopathies. Occasionally, the presence of TTP can serve as a clue to the recognition of some outbreaks of *E. coli* O157:H7 infection[117]. Although progression from hemorrhagic colitis to TTP is rare, in one outbreak up to 8% of patients with *E. coli* O157:H7 hemorrhagic colitis developed TTP[117]. Progression to TTP from hemorrhagic colitis due to *E. coli* O157:H7 has not been reported to occur in children, only in adults.

Between 2% to 7% of patients with EHEC diarrheal illness will progress to develop extraintestinal complications, most commonly HUS[6,8,14,21,125,140,146]. HUS usually develops 5 to 10 days after the onset of diarrhea[92,119]**(Figure)**. While most patients who have *E. coli* O157:H7-associated HUS will have bloody diarrhea, some will have non-bloody diarrhea[10], while others will have no prodromal diarrheal illness[117]. The severity of the gastrointestinal prodrome has been shown to reflect the severity of the extraintestinal processes and also affects the resulting long-term outcome[147]. Widespread vascular damage, often followed by permanent sequelae, is characteristic of patients with the most severe colitis[147].

Clinical Manifestations of Infection with Enterohemorrhagic *E. coli*

Clinical Presentation	Comments
Asymptomatic infection	Documented in outbreaks and household contacts
⇩ Nonbloody diarrhea	Frequently afebrile, lasts a few days
⇩ Hemorrhagic colitis	Severe abdominal cramps, bloody diarrhea, low grade or no fever
⇩ Hemolytic-uremic syndrome	Microangiopathic hemolytic anemia, thrombocytopenia and renal failure develops 1 week after diarrhea
⇩ Thrombotic Thrombocytopenic Purpura	Neurologic signs and fever usually present, generally not characterized by prodromal diarrheal illness

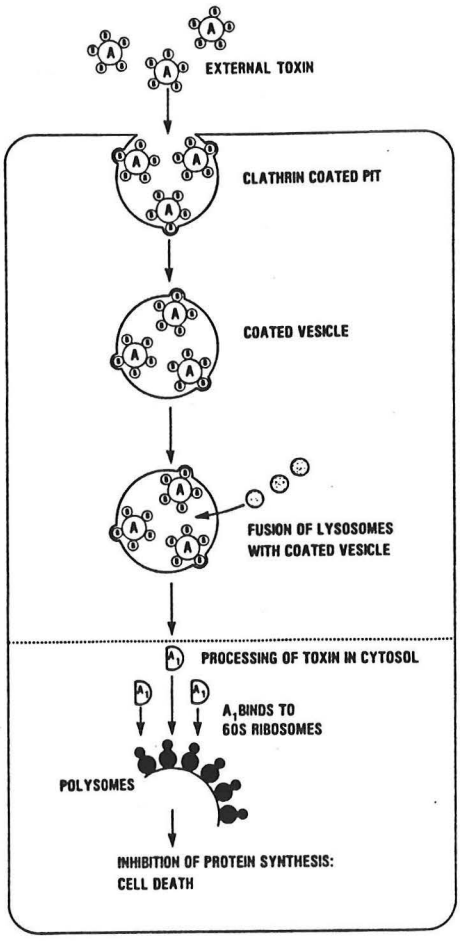
Other intestinal or extraintestinal complications that may possibly occur as part of a severe case of HUS include: cholecystitis, colonic perforation, intussusception, pancreatitis, posthemolytic biliary lithiasis, postinfectious colonic stricture, and rectal prolapse[99]**(Table)**. A severe EHEC infection, with or without HUS, may also be complicated by appendicitis, bacterial ileocecitis, hemorrhagic cystitis, and postinfectious abdominal pain[99].

Intestinal and Extraintestinal Complications of *E. coli* O157:H7 Infections

- **Hemolytic-Uremic Syndrome**
  - **Cholecystitis**
  - **Colonic Perforation**
  - **Intussusception**
  - **Pancreatitis**
  - **Posthemolytic biliary lithiasis**
  - **Postinfectious colonic stricture**
  - **Rectal prolapse**
- **Appendicitis**
- **Bacterial ileocecitis**
- **Hemorrhagic cystitis**
- **Postinfectious abdominal pain**

Pathogenesis of *E. coli*-induced HUS/TTP. While *E. coli* O157:H7 accounts for most cases of HUS in North America, in studies of children in Argentina, where the incidence of the syndrome is among the highest in the world and where meat consumption is almost universal, investigators have isolated *E. coli* O157:H7 in only 2% of children with HUS[26]. In contrast, shiga-like toxin was isolated in 48% of these children[26].The observations that HUS occurs in association with *Shigellae dysenteriae* type 1 infection[133] and with SLT-producing *E. coli* of non-O157:H7 serotypes, suggest that the common pathogenic mechanism of all of these HUS-inducing infections is related to the toxin. Indeed, the common feature among *E. coli* strains that are associated with HUS is production of significant amounts of SLT toxin. For example, evidence of SLT producing *E. coli* was detected in 75% of children with "idiopahtic" HUS, while serotype O157:H7 was identified in only 25%[113]. In a family outbreak of HUS that involved five children, SLT was isolated from each child's stools, while *E. coli* O157:H7 was isolated in only two of the five[132].

E. coli strains which elaborate SLT-II are associated with a higher risk of progession to HUS than *E. coli* isolates which produce SLT-I[141]. In a patient with HUS, it is uncommon to encounter an EHEC isolate that expresses only SLT-I, although many isloates will elaborate both SLT-I and II. Bacteriophages may infect several different *E. coli* serotypes, thereby confering the ability to cause disease.



SLTs and shiga toxin have common molecular features. Both toxins consist of five B subunits (responsible for target cell binding) and one enzymatically active A subunit. The sequence of SLT-I or SLT-II mediated toxicity begins with binding of the B subunit of SLT to specific glycolipid receptors on the surface of its target cells. There are at least two structurally-related receptors that have been identified for SLT, galabiosyl ceramide and globotriosyl ceramide, also called Gb_3[148,149]. These are the same receptors to which shiga toxin binds[150].

Internalization of the SLT-Gb_3 is accomplished by receptor-mediated endocytosis through clathrin-coated pits **(Figure)**. Once presented to the cytoplasm, the A subunit is dissociated from the B subunits. The activated A subunit inhibits protein synthesis by inactivating ribosomal subunits[151] which blocks peptide elongation[152] and leads to cell death. When cultured human renal endothelial cells are exposed to minute concentrations of shiga toxin, protein synthesis is reduced by 50% [153].

Most cell types are insensitive to SLT because they do not express Gb_3 on their surface. The organ distribution of glycolipd receptors for SLT likely determines which organs are damaged during the toxemia of *E. coli* O 157:H7 or other SLT-producing strains. For example, human glomerular endothelial cells express very high quantities of Gb_3 receptors and SLT binding in the kidney is directly proportional to Gb_3 receptor density[137]. Furthermore, in the human kidney, Gb_3 receptor concentration is higher in the cortex than in the medulla, correlating with the clinical distribution of renal lesions in HUS[137,153]. This observation may explain why the kidney is one of the most commonly affected organs in *E. coli* O 157:H7-associated HUS. Of further interest, glomerular endothelial cells from infants (children less that 2 years of age) but not from adults express Shiga-toxin receptors, suggesting that the greater frequency of HUS in very young children could be related to glomerular expression of Gb_3 early in life[154,155].

When rabbits are experimentally administered SLT-I, the organs which develop thrombotic microangiopathic injury are different from the organs commonly involved in human HUS. In rabbit HUS, there is no renal microangiopathic disease. When the distribution of Gb_3 receptors in rabbits is analyzed, the pattern of organ microangiopathic involvement parallels Gb_3 receptor distribution [156] **(Table)**. Unfortunately, since the renal endothelial cells of rabbits do not express the Gb_3 receptor, they are not a suitable animal model for HUS.

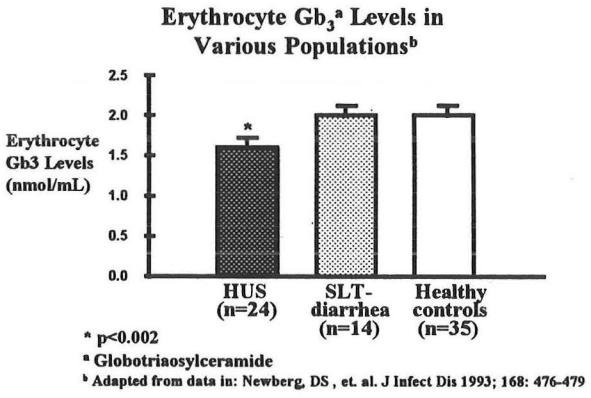
Distribution of Shiga-like Toxin Receptors Determine Sites of Injury in Rabbits Given SLT

	Verotoxin receptor	Tissue lesions
Brain	++	++
Cerebellum	++	++
Spinal cord	++	++
Colon	++	++
Kidney	-	-
Heart	-	-
Liver	-	-
Spleen	+/-	-
Lung	+	+

Remuzzi. Kidney International 1995; 47: 2-19

In mice it has been suggested that shiga toxin induces renal tumor necrosis factor (TNF) synthesis and that TNF may contribute to SLT-induced renal injury[136]. This is supported by the observation in humans that TNF induces Gb_3 receptor in endothelial cells, thereby sensitizing cells to the toxic effects of shiga toxin[157].

Red Blood Cell Antigens. Red blood cells synthesize little, if any, proteins. Therefore, binding of SLT to red cell membranes with subsequent cytoplasmic internalization of the toxin should have little effect on erythrocyte protein synthesis or on erythrocyte viability. Consequently, preferential binding of SLT to red cells rather than to epithelial cells might potentially reduce end-organ damage and ameliorate hemolysis during SLT-producing infections.



Erythrocyte Gb_3[a] Levels in Various Populations[b]

* p<0.002

[a] Globotriaosylceramide

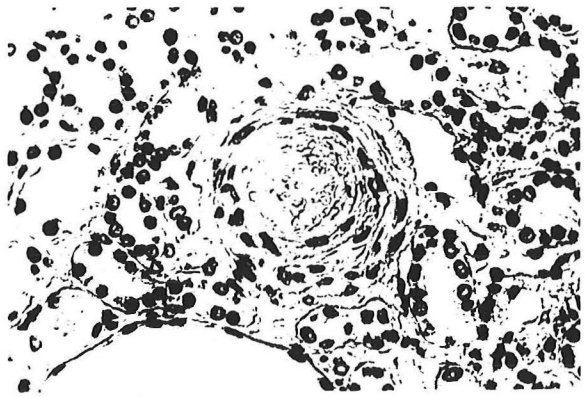
[b] Adapted from data in: Newberg, DS, et. al. J Infect Dis 1993; 168: 476-479

Erythrocyte surface Gb_3 and other glycolipid receptors vary in human populations[158]. The relationship between susceptibility to HUS and erythrocyte Gb_3 levels has been studied[159] in patients with SLT-induced diarrhea, post-diarrheal HUS syndrome & in healthy controls. As seen in the **Figure**, patients with HUS have significantly lower erythrocyte Gb_3 levels than patients with SLT-induced diarrhea, even though both were exposed to SLT[159].

Therefore, greater erythrocyte Gb_3 expression in those with SLT-induced diarrhea appears to be protective against the development of HUS and is not significantly different from erythrocyte Gb_3 expression in healthy controls. In addition to Gb_3, SLT also binds to a pentosyl ceramide molecule (P1 antigen) on erythrocytes which may also reduce competition for binding of Gb3 to renal glomerular or arteriolar endothelium[160].

The sequence of events that follows injury of renal endothelial cells by SLT *in vivo* is not well understood, mainly because of the lack of a suitable animal model of HUS. However, pathological studies of renal biopsy specimens from patients with the syndrome have provided diagnostic and prognostic information[161,162] and is discussed in the section below.

HUS Histopathology. Renal histopathology involves the glomeruli and arterioles[101,161-163]. Characteristic light microscopy findings include thickened glomerular capillary walls with expansion of the subendothelial space and narrowing of capillary lumens. Platelets accumulate as a result of their adhesion to activated endothelial cells, fibrin is deposited, indicating local generation of thrombin, all of which results in microthrombi formation in glomeruli and in extraglomerular capillaries. Glomerular ischemic changes, characterized by retraction of the glomerular tuft and marked thickening & wrinkling of capillary walls, and necrosis are occasionally found **(Figure, right)**. In severe cases, fibrinoid necrosis develops in afferent arterioles along with diffuse cortical necrosis and extensive tubulointerstitial disease. By electron microscopy, swollen glomerular endothelial cells with deposition of fibrin and lipids in the subendothelial space may be seen [101].



Although the kidney is the primary organ affected by HUS, it is not the only organ injured. Since glycolipid receptors for SLT are widely distributed in endothelium, multiorgan involvement may be pronounced in especially severe in cases of HUS/TTP. In such patients, postmortem examinations demonstrate widespread vascular lesions with platelet-fibrin microthrombi[124]**(Figure, left)**.

In the recovery phase of HUS, through local fibrinolysis and regeneration of endothelial cells, there is usually complete or partial recovery of renal function after SLT-induced HUS[164]. However, if renal function is to recover, precise control of hypertension is essential to prevent secondary vascular lesions induced by shear stress[154].

Risk Factors for Progression to HUS/TTP. Among patients who present with *E. coli* O157:H7 diarrheal illnesses, those with high temperatures or leukocytosis with a left shift on presentation[92], and very young age or very old age[92,119,138] appear to be at greatest risk for progression to HUS. The disproportionate number of HUS cases in children younger than 5 years of age suggests that host susceptibility factors may be important in selecting which patients with *E. coli* O157:H7 will progress to develop HUS[165]. While the incidence of HUS in young children is high, mortality is childhood cases is only 5 to 10%[113]. This compares to as high as an 88% mortality in elderly patients[88]. With respect to toxin production, SLT-II production is more strongly associated with HUS development than SLT- I. In the host, the primary determinant of progression to HUS in the EHEC-induced colitis is age. Most patients with HUS are either less that 5 years of age[21,135] or very elderly[88,115]. As described in the previous section, tissue distribution of Gb3 receptors is likely the cellular basis for the epidemiologic observations and for the specific organs involved in HUS. Prior gastrectomy and prior use of antimotility agents have been reported to be risk factors during outbreaks[166]. **(Table).**

Risk Factors for HUS / TTP

Bacterial Factors	Host Factors
□ SLT production	□ Very young age
□ SLT-II >> SLT-I	□ Very old age
	□ GB_3 receptors:
	Tissue distribution
	RBC GB_3 possibly protective
	□ Prior gastrectomy
	□ Use of antimotility agents

DIAGNOSIS

Routine stool cultures do not identify *E. coli* O157:H7. E. *coli* O157:H7 differs from 80% other *E. coli* in that it does not rapidly ferment D-sorbitol[13,167] and that it yields a negative result in the methyl-umbelliferyl glucuronide assay[168] which measures glucoronidase activity [169] These microbiological and biochemical characteristics have been extensively used to distinguish isolates of *E. coli* O157:H7 from other related bacteria. In sorbitol-MacConkey agar, sorbitol rather than lactose is the carbon source[170]. Since *E. coli* O157H:7 is unable to rapidly ferment sorbitol, it produces colorless colonies when grown on sorbitol-MacConkey (SMAC) agar culture plates and read at 24 hours[3,13,167].The sorbitol-negative, colorless colonies can then be screened for agglutination in O157 antiserum [171-173] or in antisera to another of its antigens[167,174].Not only has this methodology been useful for evaluation of humans for possible EHEC infections, it is also commonly implemented when assessing foods or environments as potential sources for infection [175-179].

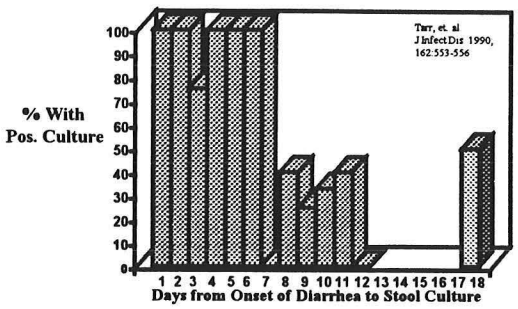
When specifically searched in investigational studies, *E. coli* O157:H7 is found to be a very common pathogen[6]. However, it is rarely isolated in clinical microbiology laboratories of community hospitals in non-investigational settings[180]. Accordingly, in the United States, relatively little information is available regarding the frequency of isolation of *E. coli* O157:H7 from patients who present with diarrheal illnesses[18]. Based on extrapolation of data from areas where rates of *E. coli* O157 infection have been prospectively assessed, it is estimated that there should be 21,000 annual cases of *E. coli* O157 infection in the United States[1]. However, during the two year period between 1994-95, only 998 cases were reported to the CDC[17]. Thus, it is very likely that there are a number of cases of *E. coli* O157:H7 that go undiagnosed by current laboratory methodology.

Up until recent years, most U.S. laboratories have not routinely cultured for this organism[180]

and, in many, cultures for *E. coli* O157:H7 must be specifically requested. However, many clinical lab have now incorporated SMAC agar as a primary screening medium for routine stool cultures[181], a practice that will undoubtedly result in greater community detection rates of *E. coli* O157:H7[7]. At the least, all patients who present with bloody diarrhea should have their stools analyzed for this pathogen.

A few features may decrease the yield of positive stool cultures for *E. coli* O157:H7. Many patients rapidly clear *E. coli* O157:H7 from their gastrointestinal tracts. Consequently, the yield of positive cultures in patients with diarrhea decreases as the interval from onset of symptoms to stool collection increases beyond six days[131]**(Figure)**. Therefore, it is critical to promptly obtain and culture stools soon after clinical disease becomes manifest. Second, in patients who excrete relatively low numbers of *E. coli* O157:H7, a small number of colorless colonies on SMAC agar may escape detection[99]. Furthermore, excretion of detectable organisms may be infrequent. Therefore, a negative stool culture does not necessarily rule out an *E. coli* O157:H7.

Effect of Timing of Stool Collection on Yield of Positive Cultures for *E. coli* O157:H7



Although genotypic studies show serotype O157:H7 to be a unique clone only distantly related to other *E. coli* serotypes[182,183], phenotypic diversity with the serogroup[184] may complicate existing laboratory procedures used to detect this pathogen in clinical specimens or in the food supply. This has obviously impacted diagnostic assays used to detect this pathogen. Although extremely useful, isolating and identifying the pathogen exclusively based on the absence of sorbitol fermentation has some limitations. Other enteric bacteria, such as *E. hermanii* and *Hafnia* species, share similar phenotypes and resemble *E. coli* O157:H7 on sorbitol-containing media. Likewise, strains of O157, of the non-H7 serotype are pathogenic and do not ferment sorbitol have been occasionally isolated from foods[185]. Therefore, because of the presence of phenotypically similar species, all sorbitol negative isolates must be serologically confirmed with O157[186] and, if available H7 antisera.

Although, the use of sorbitol containing media is intended primarily to select for *E. coli* of serotype O157:H7, sorbitol containing media may also exclude isolation of other pathogenic *E. coli* serotypes, many of which ferment sorbitol. It appears that serotype O157:H7 is the predominant pathogenic serotype worldwide. However, a large number of other serotypes are known to produce SLTs and cause mostly nonbloody diarrhea[6,115].Cases of non O157:H7 *E. coli* serotypes which cause bloody diarrhea in the United States are rare. However, a outbreak of bloody diarrhea in Montana was recently described and was felt to have been caused by a SLT-II producing strain of *E. coli* of serotype O104:H21. In contrast, in Europe there have been a number of cases of non-O157:H7 serotypes which elaborated SLTs and were associated with cases of hemorrhagic colitis and/or hemolytic uremic syndrome.[115,187].

A recent finding which has even greater implications regarding the reliance on sorbitol for pathogen identification comes from observations that *E. coli* O157:H7 isolates in sorbitol containing foods can mutate from a sorbitol non-fermenter to a sorbitol fermenter[188]. Furthermore, the frequency of isolation of sorbitol fermenting O157 strains in Europe appears to be increasing. For example, strains of *E. coli* O157:H- in Germany which produce SLT-II have been isolated from patients with HUS.[189] In contrast to serotype O157:H7, these serotypes ferment sorbitol and have positive reactions in the methyl-umbelliferyl glucuronide assay. Such strains were initially considered uncommon in Europe. However, in Central Europe, sorbitol fermenting, pathogenic serotype O157:H- strains are increasingly more common[33]. In another report, serologic and biochemical characterization of 41 SLT-producing, O157 strains (including H7 and H- serotypes) determined that as many as 25% of these isolates were sorbitol positive.

Enzyme immunoassays[190] and immunofluorescent examination of fresh stool with use of O157 antiserum have recently been proposed as a rapid techniques for detection of *E. coli* O157:H7[67,191]. Enzyme-linked immunoassays directed against SLT have also been developed[192]. If one of the rapid antigen detection tests is used, however, a confirmatory stool culture should always be performed[99].

An antibody response to the O157 lipopolysaccharide antigen can often be detected if sought in the first several months after HUS. The frequency of serological response among patients with enteric infection that does not progress to HUS is unknown. If it is important to document that *E. coli* O157:H7 is the cause of enteric infection and if the stool culture is negative, a serological test would be most appopriate[193].The various clinical laboratory techniques available for diagnostic evaluation of *E. coli* O157:H7 infection are shown in the **Table.**

Diagnosis of *E. coli* O157:H7 Infection

Culture:

- **Sorbitol-MacConkey agar culture**
  - **Serologic identification of clear colonies**

Non-Culture Diagnostic Techniques:

- **Direct immunofluorescence of stools**
- **Verocytotoxicity assay**
- **ELISA to Shiga-like toxin**
- **Serology**

Molecular Diagnostic Techniques for Identification of *E. coli* O157H:7

The problems associated with the microbiologic and serologic identification of pathogenic *E. coli* species has led to the introduction of various molecular diagnostic techniques which have facilitated the detection of this serotype and its phenotypic variants. Molecular techniques have been very useful to characterize strains[194], particularly during epidemiologic investigations of outbreaks[17,40]. Restriction fragment length polymorphism (RFLP)[189,194] and pulse field gel electrophoresis (PFGE)[17] have helped distinguish toxigenic from non-toxigenic strains and have been useful in detecting infections not detected by traditional methods. Polymerase chain reaction (PCR) has been used to amplify genes of SLT in stool samples or bacterial isolates[195-198]. This technique has been especially useful for rapid analysis of large sample numbers.

THERAPY

Since *E. coli* O157:H7 infections can present with a wide range and severity of clinical manifestations, possible therapeutic approaches are discussed according to the specific manifestation.

Diarrheal Illness. Patients with *E. coli* O157:H7 diarrheal (bloody or nonbloody) illnesses present a compelling case for antibiotic therapy. They are in severe pain caused by an organism that is usually susceptible to a wide range of antibiotics. However, antibiotic treatment is not recommended as they have not been found to alter the course of the disease. In retrospective analyses, patients who received antibiotics were found to have the same or greater risk of developing HUS as those who received no antimicrobial agents[21,92]. In a prospective study in which trimethoprim-sulfamethoxazole was administered late after the onset of symptoms, neither benefit nor harm was apparent in the treated group[199]. Furthermore, patients have contracted *E. coli* O157:H7 infection during antibiotic therapy for other conditions, and, despite sensitivity of their *E. coli* isolate to the antibiotic administered, their courses have not been measurably less severe. In fact, prior antibiotic use has been identified as a risk factor for a poor outcome from *E. coli* O157:H7 infection[88]. Unfortunately there have been no prospective, randomized controlled trials of the safety and efficacy of antibiotic treatment.

The initial management of the enteritis should be directed at maintenance of the fluid and electrolyte balance. Transfusions of packed red blood cells should be provided as necessary. The use of antimotility agents is associated with retention of Shiga toxin and a greater risk of HUS/TTP and more severe neurological manifestations[200]. Therefore the use of antimotility agents in confirmed or suspected cases of *E. coli* O157:H7 colitis is contraindicated. The efficacy of toxin-binding agents is currently under clincial trial.

A complete blood count, smear analysis and platelet count should be performed four days after the onset of diarrhea to assess for the possiblity of HUS[99]. Because confirmed culture results are usually not available at time of presentation of patients with bloody diarrhea, colonoscopy should be considered to rule out other causes of hematochezia.

HUS/TTP Syndrome. When an overt HUS or TTP syndrome occurs in conjuntion or after a SLT-induced diarrheal illness careful attention to management of the renal failure should be given and dialysis is commonly necessary. Plasma infusion or plasma excahnge is recommended when more conservative measures have failed[124,129]. The results with plasma infusion in children, however, have not been shown to significantly improve outcome[124].

PREVENTION

The CDC, in collaboration with the U.S. Department of Agriculture's Food Saftey Inspection Service, has identified critical control points in meat processing to reduce the likelihood of pathogens such as *E. coli* O157:H7 entering the meat supply. There is extensive amount of ongoing veterinary research to reduce bovine carriage rates of EHEC. Since ground beef is likely to be internally

contaminated, the optimal food protection practice is to cook ground beef thoroughly until the interior is no longer pink and the juices are clear. The Food and Drug administration has therefore issued recommendations to increase the internal temperature for cooked hamburgers to 155°F (86°C). As discussed earlier, apple cider and juice and milk should be pasteurized.

The dose of *E. coli* O157:H7 that leads to symptomatic infection is low[81]. For this reason, patients known to be infected by EHEC should be considered highly contagious and should not return to group settings unless hygenic practices are maintained [99]. For children who are in day care settings, some have suggested that at least two negative stool cultures be obtained prior to return to day care[86].

Case detection rate might be increased by specifically requesting from the lab diagnostic tests for *E. coli* O157:H7. All cases of confirmed *E. coli* O157:H7 infection should be reported immediately to health authorities. Cases of HUS and clusters of nonbloody diarrhea should also be reported, even if *E. coli* O157:H7 has not been proven[99]. Early detection of clusted cases can trigger timely epidemiologic investigations and may avert continued transmission.

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