Escherichia coli O157:H7 — A Meatborne Pathogen of Serious Consequences

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Escherichia coli O157:H7 was first identified as a human pathogen in 1982 when the bacterium was associated with two outbreaks of hemorrhagic colitis. Ground beef was epidemiologically linked as the vehicle of transmission. Subsequently, several more outbreaks have been reported in the United States, Canada and England (Padhye and Doyle, 1992; Tauxe and Griffin, 1991). Undercooked ground beef has been identified as the most frequent vehicle of infection, with raw milk, prepared sandwiches, potatoes, apple cider, roast beef and water also implicated as vehicles.

Modes of Transmission

A large four-state outbreak involving more than 500 cases and 4 deaths occurred in 1993. Ground beef sandwiches, which were cooked to an internal temperature of 60°C (140°F) or less, were the vehicle of infection. Enumeration studies revealed that frozen beef patties of the implicated lots contained <15 *E. coli* O157:H7 per gram, suggesting that the infectious dose of the pathogen is low. The apparent ease of acquiring illness by person-to-person transmission further supports the likelihood that the infectious dose of *E. coli* O157:H7 is low. Several outbreaks have occurred in day-care centers and in institutional settings such as nursing homes in which person-to-person transmission was documented. Municipal water also was identified as a vehicle of transmission in an outbreak involving approximately 240 residents of Cabool, Missouri, illustrating that the organism can be spread by contaminated water. There is seasonal variation in the occurrence of *E. coli* O157:H7 in the U.S. and Canada, with the highest incidence occurring during the summer months of June through August.

Manifestations of Illness

Symptoms of illness caused by *E. coli* O157:H7 range from mild to severe. Mild diarrhea or asymptomatic infection has been reported; however, hemorrhagic colitis (HC) is the most frequently reported manifestation of illness. HC usually begins with a sudden onset of severe abdominal cramping, often described as mimicking labor pains or appendicitis. This is followed within 24 hours by watery diarrhea which later becomes grossly bloody described as "all blood and no stool."

Illness in some (about 5%) patients, especially children and the elderly, progresses to a more severe syndrome, hemolytic uremic syndrome (HUS). Clinically, patients with HUS appear seriously ill or sometimes jaundiced and often hypertensive. Red blood cells within capillaries of the kidneys and other organs clot, resulting in an accumulation of waste products in the blood. Kidneys become dysfunctional, thereby necessitating dialysis and blood transfusions. Cardiovascular and central nervous system disorders occur resulting in heart failure, seizures, coma and death.

The most severe manifestation of *E. coli* O157:H7 infection, thrombotic thrombocytopenic purpura (TUTP), occurs infrequently but has a high mortality rate. This syndrome occurs principally in adults and is similar to HUS except the central nervous system is principally involved. Patients often develop blood clots in the brain causing fluctuating neurologic signs and often death.

Reservoirs

Cattle have been identified as an important reservoir of *E. coli* O157:H7. Surveys of cattle of dairy herds revealed that calves and heifers more frequently carry the organism than adults. The pathogen is isolated from feces, and apparently colonizes the intestinal tract. The bacterium also has been isolated from feces of beef cattle. Studies of calves with diarrhea revealed that *E. coli* O157:H7 is seldom associated with these animals, indicating that the organism is not a likely cause of diarrheal disease in cattle. A 1991 national survey of dairy cattle for carriers of *E. coli* O157:H7 revealed that 0.36% of cattle shed the organism in feces and about 5% of herds were positive. A more recent survey, using more sensitive isolation procedures, has found 22% of herds and 2.9% of calves positive for the pathogen.

Presence in Foods

Very few studies have been done to determine the prevalence of *E. coli* O157:H7 in foods; however, surveys of retail meats from grocery stores in Madison, Wisconsin, detected the organism in 1% to 3% of ground beef and 1% to 2% of poultry, pork and lamb samples. The pathogen also has been isolated from raw milk produced in Wisconsin or Georgia, and in venison from the state of Washington.

Toxins

The mechanism by which *E. coli* O157:H7 causes illness has not been fully defined, but an important factor associated

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with the organism's virulence is the production of one or more verotoxins. These toxins are very similar to the toxin produced by *Shigella dysenteriae* type 1 (Shiga toxin) and, because of these similarities, have been named Shiga-like toxins (SLT). Several different SLT produced by *E. coli* which share substantial amino hemology have been identified; however, only two toxins, SLT I and/or SLT H, are known to be produced by *E. coli* O157:H7. These SLT possess two subunits, A and B, that are the active and binding portions of the toxins, respectively. The B subunits bind to specific glycosphingolipids, globotriosyl ceramide (Gb3), which are receptors on the surface of cells of specific body tissues. Gb3 is abundant in the cortex of the human kidney, which is the principal site of damage in HUS. Both SLT have the same mechanism of action, which involves blocking protein synthesis by inhibiting elongation factor-I-dependent aminoacyl binding of t-RNA to 60S ribosomal subunits. This is the same mechanism of action of ricin, a cytotoxic protein present in castor beans.

Non O157 SLT-Producing *E. coli* Causing HC and HUS

Besides *E. coli* O157:H7, other *E. coli* serotypes also have been shown to produce SLT. Several of these SLT-producing non-O157 *E. coli* have been associated with patients with HUS, hence it appears that SLT-producing *E. coli* other than serotype O157:H7 are important in human disease. Examples include serotypes 026:H11 and 0111 which are also enterohemorrhagic, causing HC and HUS. However, *E. coli* O157:H7 is the most common serotype that causes HC or HUS in humans.

Unusual Biochemical Reactions of *E. coli* O157:H7

Most biochemical reactions of *E. coli* O157:H7 are typical of *E. coli*, with the exception of sorbitol fermentation and B-glucuronidase activity. Most *E. coli* isolates of human origin ferment sorbitol within 24 hours; however, typical strains of *E. coli* O157:H7 recently isolated from feces do not. Additionally, about 93% of *E. coli* strains possess the enzyme B-glucuronidase that is the basis for development of a rapid fluorogenic assay for *E. coli*. This assay uses 4-methylumbelliferyl ß-D-glucuronide (MUG) as an indicator which is hydrolyzed to a fluorogenic product by the enzyme ß-glucuronidase. *E. coli* O157:H7 typically is MUG assay negative, indicating that ß-glucuronidase activity is not phenotypically expressed by these organisms. These unique properties are used by microbiologists to isolate *E. coli* O157:H7 from clinical specimens plated on selective agar medium such as MacConkey sorbitol agar.

Detection and Isolation of *E. coli* O157:H7 in Foods

Several methods have been developed for the detection and isolation of *E. coli* O157:H7 in foods. Many procedures used to enumerate and isolate fecal coliforms and subsequently *E. coli* use incubation temperatures in the range of 44° to 45°C. However, studies have revealed that *E. coli* O157:H7 grows poorly or not at all in this temperature range. Hence, the traditional method for detecting and isolating *E. coli* from food is not suitable for detecting and isolating *E. coli* O157:H7.

A specific, sensitive, rapid and easy-to-perform procedure for detecting *E. coli* O157:H7 in foods was developed by Padhye and Doyle (1991) using a monoclonal antibody (MAb 4E8 C12) specific for *E. coli* O157:H7 and 026:H11. The entire procedure takes less than 20 hours to complete. The procedure involves enrichment of a food sample in a selective enrichment broth for 16 to 18 hours at 37°C with agitation. Enrichment culture is applied to a sandwich ELISA procedure or a dipstick assay that has a polyclonal antibody specific for *E. coli* O157:H7 as the capture antibody and MAb as the detection antibody. The sensitivity of the procedure is 0.2 *E. coli* O157:H7 per gram of food after overnight enrichment. The bacterium can be isolated from *E. coli* O157:H7-positive enrichment cultures by plating cultures onto MacConkey sorbitol agar containing MUG and selecting colonies that are sorbitol-negative and MUG-negative for biochemical and serological confirmation.

Resistance of *E. coli* O157:H7 to Acid

E. coli O157:H7 has unusual resistance to acid compared to many other enteric microorganisms and gram-positive foodborne pathogens. For example, studies of the fate of *E. coli* O157:H7 in apple cider at pH 3.6-4.0 and held at 8°C revealed that *E. coli* O157:H7 initially present at 10^2 per ml survive for up to 2 weeks. Studies of the manufacture and storage of fermented sausage revealed that *E. coli* O157:H7 populations are reduced by about 2-fold after 13 hours of fermentation to pH 4.8, by another 10-fold after 21 days of drying and a reduction of pH to 4.5, and by another 10-fold during storage in vacuum packages at 4°C for 8 weeks. There was approximately a 100-fold *E. coli* O157:H7/g reduction during the entire manufacturing and storage time. Similar studies with *Listeria monocytogenes* revealed that listeriae were decreased to less than 1 *L. monocytogenes* per gram (> 10^4 listeriae/g reduction) during the same manufacturing and storage conditions. Hence, *L-monocytogenes* is considerably more sensitive to the acidic and environmental conditions of fermented sausage than *E. coli* O157:H7.

Carcass washing of beef with acid sprays recently has been promoted as a means of reducing the prevalence of pathogens on meat. Recent studies have revealed that *E. coli* O157:H7 on beef is exceptionally tolerant to treatments of 0.5, 1.0 or 1.5% citric, lactic, or acetic acid at 22° or 55°C and then held at 4°C for up to 13 days. For all treatments, less than a 0.5 log *E. coli* O157:H7 per gram reduction was observed. Many gram-positive bacteria such as *Staphylococcus aureus* or *L. monocytogenes* are more sensitive to these treatments than *E. coli* O157:H7.

Summary

E. coli O157:H7 is now recognized as an important human pathogen. Illnesses caused by this pathogen can range from self-limited, watery and bloody diarrhea to life-threatening

manifestations such as HUS or TTP, which can result in death. The mode of transmission is primarily through food; however, person-to-person transmission also has been identified in some institutional settings. Studies to date indicate that cattle are an important reservoir of the organism.

References

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