Escherichia coli Serotype O157:H7: Novel Vehicles of Infection and Emergence of Phenotypic Variants

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Escherichia coli *serotype O157:H7 was only recognized as a human pathogen a little more than a decade ago, yet it has become a major foodborne pathogen. In the United States, the severity of serotype O157:H7 infections in the young and the elderly has had a tremendous impact on human health, the food industry, and federal regulations regarding food safety. The implication of acidic foods as vehicles of infection has dispelled the concept that low-pH foods are safe. Further, the association of nonbovine products with outbreaks suggests that other vehicles of transmission may exist for this pathogen. In laboratory diagnosis, most microbiologic assays rely on a single phenotype to selectively isolate this pathogen. However, the increasing evidence that phenotypic variations exist among isolates in this serogroup may eventually necessitate modifications in assay procedures to detect them.*

Enterohemorrhagic *Escherichia coli* (EHEC) has emerged in recent years as the predominant cause of hemorrhagic colitis in humans. This illness, with characteristic symptoms of bloody diarrhea and abdominal cramps, can progress into a more severe, life-threatening complication known as hemolytic uremic syndrome (HUS). The pathogenicity of EHEC appears to be associated with a number of virulence factors, including the production of several cytotoxins (1,2). These toxins are collectively referred to as verotoxins or Shiga-like toxins (SLTs) because the SLT-I of *E. coli* closely resembles the Shiga toxin of *Shigella dysenteriae* type 1 (2). Although more than 60 *E. coli* serotypes produce SLTs (2) and more are being identified as capable of producing SLT, serotype O157:H7 is the predominant pathogen in the EHEC group and the one associated most frequently with human infections worldwide.

Isolates of the serotype O157:H7 were first implicated in foodborne illness in 1982; in the subsequent 10 years, approximately 30 outbreaks were recorded in the United States (1). In early 1993, however, serotype O157:H7 received considerable attention after a large foodborne disease outbreak, traced to the consumption of undercooked, contaminated hamburgers served at a regional fast-food restaurant (3). More than 700 persons in four states were infected; there were 51 cases of HUS and four deaths. Since that outbreak, the reported incidence of serotype O157:H7 infections has risen, partly because better surveillance systems have been implemented and awareness has increased among physicians, clinical microbiologists, and consumers. Fifteen additional outbreaks were recorded in 1993 and 20 in the first half of 1994.

Because serotype O157:H7 has only recently been recognized as a foodborne pathogen, our knowledge is limited. However, the notoriety of recent outbreaks and the severity of serotype O157:H7 infections have stimulated research on the organism, its ecology, antibiotic resistance properties, and virulence factors. Much has already been learned from the epidemiologic investigations of past outbreaks. For instance, foodborne infections of serotype O157:H7 have most often been associated with the consumption of bovine products; however, several recent outbreaks have implicated other less likely vehicles of infection and showed that the organism may have some unsuspected characteristics. Although genotypic studies show serotype O157:H7 to be a unique clone only distantly related to other *E. coli* serotypes (4,5), phenotypic diversity within the serogroup (6) may complicate existing laboratory diagnosis procedures. The introduction of various molecular diagnostic techniques may facilitate the detection of this serotype and its phenotypic variants.

This review examines unexpected and seemingly unlikely vehicles implicated in recent serotype O157:H7 outbreaks and the impact of emerging phenotypic variants and their effect on diagnostic assays used to detect this pathogen in clinical specimens or in the food supply.

Novel Vehicles of Transmission

So far, serotype O157:H7 has caused a total of 60 outbreaks of foodborne illness in the United States. Consumption of contaminated, undercooked

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ground-beef products has accounted for most outbreaks; however, raw milk was also implicated in several outbreaks in the United States and Canada. Improper hygiene with secondary spread from person-to-person contact is another well-documented route of infection (1,2). In the last few years, however, several foodborne outbreaks of serotype O157:H7 have implicated unique and seemingly unlikely vehicles of infection: among them are acidic foods, fruits, salad vegetables, yogurt, and water.

Acidic foods

In the *Retail Food Store Sanitation Code* of the U.S. Food and Drug Administration, foods with a pH value of less than 4.6 are generally regarded as low risk in terms of food safety. However, several recent disease outbreaks attributable to serotype O157:H7 have shown that this pathogen can persist in foods with low pH.

In the fall of 1991, an outbreak of serotype O157:H7 that affected 23 persons was traced to the consumption of fresh-pressed apple cider (7). The implicated cider, made from unwashed "dropped" apples at a farm, had a pH value of 3.7 to 3.9, was not pasteurized, and contained no preservatives. Although apple cider had been implicated in a previous outbreak of *Salmonella typhimurium*, it is not a common vehicle of enteric infection because of its high acidity. Several laboratory studies have subsequently demonstrated that isolates of serotype O157:H7 can tolerate acidic conditions. Some strains persist in media with pH values as low as 2.0 (8), and in cold (8°C) apple cider for 10 to 31 days (7,9). Although the source of serotype O157:H7 in the cider that caused illness was never fully established, it was suspected that the dropped apples had been contaminated by cow manure.

The ability of serotype O157:H7 to tolerate acidity was substantiated in 1993, when another acidic food was implicated in a series of restaurant outbreaks that infected at least 48 persons. Although the source of the outbreaks was not conclusively identified, epidemiologic investigations and other data implicated mayonnaise or mayonnaise-based dressing and sauces. Samples of mayonnaise had a pH of 3.6 to 3.9, and the sauces prepared from it were also acidic, with pH levels of 3.6 to 4.4 (10). After this outbreak, several studies confirmed that although isolates of serotype O157:H7 do not multiply under these conditions, they can persist in commercial mayonnaise up to 55 days at 5°C (10,11). How the mayonnaise became contaminated with serotype O157:H7 was not determined; however, improper handling of bulk mayonnaise or cross-contamination with meat juices or meat products was suspected.

Water

Several recent incidents show that both drinking water and recreational water can serve as vehicles for transmitting serotype O157:H7 infections.

The first and largest waterborne outbreak associated with this pathogen occurred in Missouri in 1989 (12). Of the more than 240 people infected, 32 were hospitalized, and four died. The source of the outbreak was not identified, but backflow during a water main break might have contaminated the drinking water supply (12). Like most *E. coli*, serotype O157:H7 isolates are susceptible to the effects of chlorine. Hence, adjustments in the chlorination of the drinking water supply during repairs to the water main might have prevented the outbreak (12).

An outbreak caused by serotype O157:H7 and *S. sonnei* in 1991 may have involved recreational lake water in the vicinity of Portland, Oregon. Of the 59 people affected, 21 (all children) were infected by serotype O157:H7 (13). An epidemiologic survey showed that those who became ill had swum in the lake during the previous 3-week period. Transmission probably occurred when the swimmers swallowed lake water that was fecally contaminated by other bathers. The lengthy period during which people became infected suggests that these pathogens can remain viable in water for a long time, or that the water was repeatedly recontaminated. Fecal contamination of recreational water by bathers, especially small children, is not uncommon; however, the contaminants are usually diluted quickly by the large volume of water in recreational lakes, bays, or rivers. That swallowing a small amount of lake water can cause illness suggests that the pathogen has a low infectious dose (13). This fact is already well established for *Shigella* and seems to be consistent with recent epidemiologic data from foodborne outbreaks associated with serotype O157:H7.

A similar incident, implicating water from a children's paddling pool, was reported in Scotland in 1992 (14). Although epidemiologic evidence was not conclusive, the available data suggested that a child with diarrhea had played in the pool and fecally contaminated the water with serotype O157. Because the pool water was not changed or disinfected, it became the vehicle of infection for two other neighborhood children, who in turn infected others by person-to-person contact.

Other vehicles of transmission

Recently, several other unique vehicles have been implicated in foodborne outbreaks associated with serotype O157:H7. A 1993 outbreak in an Oregon restaurant was apparently caused by the consumption of cantaloupe or other items from the salad bar, which were most likely cross-contaminated by meat products during preparation. One study showed that

serotype O157:H7 can survive and grow on salad vegetables stored at 12°C and 21°C for up to 14 days (15). An outbreak in the United Kingdom in 1991 was traced to the consumption of yogurt, which infected 16 persons, 11 of them children (16). Although consumption of raw milk has caused past outbreaks, serotype O157:H7 is susceptible to heat treatment and thus does not usually survive the pasteurization process. Even though the implicated yogurt was prepared from pasteurized milk, the milk might have become contaminated with serotype O157:H7 after pasteurization.

A puzzling incident was reported from northern Italy, where 15 cases of HUS, caused by serotype O157 and other EHEC serotypes, was recorded over a 5-month period in 1993 (17). These cases occurred in small towns scattered over a large area with little apparent connection to each other; therefore, common food vehicles and exposure to cattle were eliminated as possible sources of infection. However, data from the epidemiologic investigations suggested that contact with live poultry or with chicken coops may have been the source of infection, even though no toxin-producing EHEC strains were isolated from poultry feces. A recent study showed that inoculating 1-day-old chicks with strains of serotype O157:H7 resulted in rapid colonization of the cecal tissue of the chicks. The chicks then became long-term (up to 11 months) shedders of serotype O157:H7, and this microorganism was subsequently recovered from the shells of their eggs (18). It is conceivable, therefore, that live poultry were the source of infection in the outbreaks reported from northern Italy.

In December 1994, dry cured salami was implicated as the source of serotype O157:H7 in a disease outbreak in the state of Washington (19). A prior study showed that although isolates of serotype O157:H7 do not grow in seeded sausage batter, they can tolerate the acidity produced during sausage fermentation and survive the drying and the cold storage associated with the preparation of dry sausages (20). Fermented sausages can attain a pH as low as 4.8 (20). The ability of serotype O157:H7 isolates to persist under these conditions is consistent with the acid-tolerant properties this organism exhibited in the previously discussed studies with apple cider (7) and mayonnaise (10).

Although the consumption of bovine products still accounts for most of the serotype O157:H7 infections, the incidents described above show that other food types can also serve as vehicles in transmitting infections with this serotype.

Emergence of Phenotypic Variants

Multilocus enzyme electrophoresis of *E. coli* strains associated with enteric disease show that serotype O157:H7 is in a well-defined group only distantly related to other SLT-producing serotypes (4,5). Recently, however, several phenotypic variants of this serotype were isolated in Europe. Thus, in addition to causing infections through food vehicles, the problems associated with serotype O157:H7 are compounded by the emergence of phenotypic variants, which may have an impact on diagnostic assays used to detect this pathogen.

The clonal nature of serotype O157:H7 has facilitated its phenotypic identification. Unlike other *E. coli*, isolates of serotype O157:H7 do not ferment sorbitol in 24 hours (21) and are negative in the methyl-umbelliferyl glucuronide assay (22), which measures glucuronidase activity (23). These phenotypes, especially the absence of sorbitol fermentation, are used extensively to distinguish isolates of serotype O157:H7 from related bacteria. Isolation of serotype O157:H7 from foods, on selective media, such as hemorrhagic colitis agar (24) and cefixime-tellurite sorbitol-MacConkey agar (25) is based on the sorbitol phenotype. Similarly, sorbitol-MacConkey agar (26) is used in the clinical laboratory as the primary screening medium to analyze patient specimens for the presence of serotype O157:H7. Prompt culturing of bloody stools with this agar has been very effective in isolating serotype O157:H7 from stool specimens (1).

Although extremely useful, isolating and identifying the pathogen exclusively on the absence of sorbitol fermentation has some limitations. Other enteric bacteria, such as *E. hermanii* and *Hafnia* spp., share similar phenotypes and resemble serotype O157:H7 on sorbitol-containing medium. Likewise, strains of O157, of non-H7 serotype that are not pathogenic and do not ferment sorbitol have occasionally been isolated from foods (27). Because of the presence of phenotypically similar species sorbitol negative isolates must be serologically confirmed with O157 and H7 antisera (28).

Though intended solely to select for serotype O157:H7, sorbitol-containing media may also exclude the 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 also produce SLT (1,2). Although many of these have not been implicated in disease or are known to cause only nonbloody diarrhea, some reports indicate that selected SLT-producing, non-O157:H7 serotypes may have caused cases of hemorrhagic colitis and HUS in Europe (29,30). In the United States, disease caused by non-O157:H7 serotype is rare; however, a recent outbreak of bloody diarrhea in Montana was suspected to have been caused by a SLT-II-producing *E. coli* of serotype O104:H21 (31).

A more relevant finding, and one that has stronger implications regarding the reliance on sor-

bitol phenotype for identifying pathogens, comes from a recent study which showed that isolates of serotype O157:H7 in sorbitol-containing foods can mutate from a sorbitol-nonfermenting to a sorbitol-fermenting phenotype (32). Moreover, the frequency of isolation of sorbitol-fermenting O157 strains in Europe appears to be increasing. In Germany, for instance, strains of serotype O157:H$^-$ that produce SLT-II have been isolated from HUS patients (33). Unlike serotype O157:H7, these strains fermented sorbitol and were positive in the methyl-umbelliferyl glucuronide assay. Initially, these strains were considered atypical. However, other studies confirmed that pathogenic, sorbitol-fermenting, serotype O157:H$^-$ strains were fairly prevalent in HUS patients in central Europe (34). 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 the isolates were sorbitol positive. Furthermore, there was considerable variation among pathogenic serotype O157 isolates not only with respect to sorbitol fermentation, but also with respect to other phenotypic characteristics (6). These variants are not detected by sorbitol-containing media and may not be identified by the routine biochemical tests used to characterize serotype O157:H7.

The notoriety of recent outbreaks has stimulated the development of many new assays to detect serotype O157:H7; some of them may also be useful for detecting phenotypic variants. Many of these assays use molecular techniques, and some are commercially available. Several new molecular subtyping methods have also been introduced. Although typing methods will not be discussed here, such techniques as ribotyping, pulsed-field gel electrophoresis (35), lambda restriction fragment length polymorphism (36), and others have been extremely useful in studying the epidemiology of serotype O157:H7 in foodborne outbreaks.

Phenotypic variants of serotype O157:H7 retain the O157 antigen; hence, antibodies to O157 antigen can be used to identify both serotype O157:H7 and its variants. In the clinical laboratory, anti-O157 sera are used effectively in agglutination or latex agglutination tests to rapidly screen or serologically confirm isolates. Some anti-O157 antibodies have also been coupled to magnetic beads and used to selectively isolate this pathogen from foods (37) or have been incorporated into enzyme immunoassays to directly detect serotype O157:H7 in foods and clinical specimens. The latter application of anti-O157 sera, however, has had some drawbacks. Many preparations of anti-O157 sera cross-react with other bacteria, including *Citrobacter freundii* (38), *E. hermanii,* and *Yersinia enterocolitica* O:9 (39). Moreover, the O157 antigen is present on other non-H7 *E. coli* serotypes (6,40), many of which are not pathogenic. For example, when anti-O157 serum was used in an analysis of various food products, nonpathogenic O157 isolates were found that neither produced SLT nor were of the H7 serotype (27). Therefore, positive test results of food samples tested with assays that use anti-O157 sera should be confirmed by other methods. Pre-absorption of diagnostic antisera to remove cross-reacting antibodies or the use of antibodies specific for other non-O157 surface antigens of serotype O157:H7 may reduce the frequency of serologic cross-reactions (41).

Phenotypic variants also appear to retain the pathogenicity of serotype O157:H7 (6,33); therefore, assays specific for virulence factors are not affected by the phenotypic variations described above. For example, anti-SLT antibodies can be used to screen fecal specimens for toxins, and SLT gene-specific DNA probes and polymerase chain reaction (PCR) can be used to identify all SLT-producing pathogens regardless of phenotype. However, assays specific for SLT or SLT genes do not provide sufficient data for epidemiologic investigations and "trace-back" studies. More than 60 *E. coli* serotypes have been found to produce SLT (1,2), and even strains from more distantly related genera, such as *C. freundii*, reportedly produce SLT-II-like cytotoxins (42). Many of these SLT-producing *E. coli* serotypes have not been implicated in disease; therefore, the mere detection of potential SLT-producing strains in foods or in patients' specimens by these assays is not conclusive evidence that the bacteria caused the illness.

Some new assays do not have these limitations. One PCR assay, designed as a mismatch amplification mutation assay, preferentially amplifies an allele in the *uid*A gene that is unique only to serotype O157:H7, including its phenotypic variants of serotype O157:H$^-$ that are sorbitol and methyl-umbelliferyl glucuronide positive (43). Coupled with primers specific for SLT genes, this multiplex PCR assay can simultaneously identify isolates of serotype O157:H7 and the type of SLT they encode (44). Analysis of pure culture isolates showed that this assay detected all SLT-producing serotypes and was able to distinguish isolates of serotype O157:H7, including the phenotypic variants.

Advantages of these new molecular methods include specificity, sensitivity, and the ability to detect phenotypic variants of serotype O157:H7. However, these assays are far too complex and costly for use in the routine analysis of food or clinical specimens. Furthermore, although the emergence of phenotypic variants is of concern, they have only been observed sporadically and are not prevalent worldwide. Nonetheless, should the frequency of isolation or the incidence of infection caused by these variants increase or should other SLT-producing serotypes of *E. coli* become more firmly established as causative

agents of illness, other media or assays may need to be incorporated into existing diagnostic methods. In the interim, the continued use of a sorbitol-containing medium such as sorbitol-MacConkey agar to screen bloody stool specimens is a useful and economical laboratory procedure for the early diagnosis of serotype O157:H7 infections.

Conclusions

Bovine products have most often been implicated in foodborne infections with *E. coli* serotype O157:H7. However, recent outbreaks indicate that other food types may also serve as vehicles of transmission for this pathogen. Most notably, acidic foods that were once thought to be of low risk can no longer be considered safe because of the acid-tolerant properties of this bacterium. Most microbiologic media and diagnostic assays have been designed specifically to detect serotype O157:H7. However, there is phenotypic diversity within the serogroup. Should these variants become more prevalent in infections, the use of sorbitol medium alone may become inadequate in detecting the diversity of strains in this pathogenic serotype.

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