Verotoxigenic *Escherichia coli* Infection: U.S. Overview

PHILLIP I. TARR, THOMAS E. BESSER, DALE D. HANCOCK, WILLIAM E. KEENE, AND MARCIA GOLDOFT

1Division of Gastroenterology, Children's Hospital and Medical Center and Departments of Pediatrics and Microbiology, University of Washington School of Medicine, Seattle, Washington 98105; 2Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, Washington 99164; 3Field Disease Investigative Unit, Washington State University, Pullman, Washington 99164; 4Acute and Communicable Disease Program, Oregon Health Division, 800 NE Oregon Street, Suite 772, Portland, Oregon, 97232; and 5Washington State Department of Health, Communicable Disease/Epidemiology Section, Seattle, Washington 98155

(MS# 96-507: Received 30 December 1996/ Accepted 26 July 1997)

ABSTRACT

*Escherichia coli* O157:H7 remains a public health problem in the United States despite a dramatic increase in the awareness of, and concern about, foodborne infections since the 1993 multistate *E. coli* O157:H7 epidemic. Although surveillance data can be difficult to interpret, the incidence of endemic disease caused by this organism is probably not increasing, and might be decreasing, at least in selected populations. With increased recognition of *E. coli* O157:H7 infection has come the investigation of increasing number of outbreaks, leading to the recognition of many "new" vehicles, including some foods not traditionally associated with enteric infections, such as dry-cured salami and lettuce. Molecular fingerprinting techniques are being used to track the transmission of *E. coli* O157:H7 through human populations. Analysis of DNA encoding virulence factors and surface antigens suggests that diarrheaegenic *E. coli* have evolved by acquiring large DNA fragments, with subsequent chromosomal recombination. Some Shiga toxin-producing *E. coli* other than *E. coli* O157:H7 are no doubt pathogens, but the majority of these toxigenic strains found in food are probably not virulent. More research is needed to define the characteristics that render selected Shiga toxin-producing organisms harmful to humans.

Key words: *E. coli* O157:H7, hemolytic uremic syndrome, Shiga toxin, verocytotoxin

In late 1992 and early 1993, a very large outbreak of *E. coli* O157:H7 infections occurred in Washington and several other western states. This outbreak, traced to undercooked hamburgers served at multiple outlets of the same fast food chain (10), placed food safety in general, and *E. coli* O157:H7 in particular, into public, industrial, and regulatory prominence. This review will focus on developments and publications from the United States pertaining to *E. coli* O157:H7 and other Shiga toxin (Stx)-producing *E. coli* between that watershed outbreak and mid-1996 that are relevant to food safety professionals.

In this review, the newly proposed nomenclature for Stx-producing *E. coli* will be used (18). Stx-producing *E. coli* are synonymous with verotoxigenic *E. coli* (29).

RECENT EPIDEMIOLOGY OF *E. COLI* O157:H7 INFECTIONS

Since the 1993 epidemic, an increasing number of *E. coli* O157:H7 infections, both sporadic and outbreak associated, have been reported in the United States. However, data from several sources suggest that the incidence of *E. coli* O157:H7 infection is not increasing. In Oregon and Washington, where there has been long-standing and widespread awareness of *E. coli* O157:H7 infections and screening for this pathogen is relatively common, the number of reported cases of *E. coli* O157:H7 infection has fallen somewhat since 1993 (Figure 1).

Surveillance tallies of *E. coli* O157:H7 infections reflect the interacting probabilities that (i) patients will seek medical attention, (ii) the appropriate culture will be performed, and (iii) diagnosed infections will be reported to public health authorities. The incidence of childhood hemolytic uremic syndrome (HUS) might be a more reliable indicator of *E. coli* O157:H7 disease incidence. *E. coli* O157:H7 has been (24, 36, 38, 52), and remains (see below), the nearly exclusive precipitant of postdiarrheal HUS in this country. HUS is unlikely to remain undetected because such patients are quite ill and almost always require hospitalization. The diagnosis is easily made by laboratory tests that are available to all physicians, and most patients are referred to relatively few specialists in a limited number of tertiary pediatric centers. Therefore, although only approximately 10% of patients with clinically apparent *E. coli* O157:H7 infections develop HUS, surveillance of pediatric hospitals to enumerate patients with HUS can indicate the frequency of human infection with this organism.

The incidence of HUS in King County, Washington (50), and Minnesota (36) increased through the 1970s and 1980s. In contrast, a recent study (47) suggested that the
approximately 6 weeks (20). E. coli O157:H7 has also been isolated from other ruminant species, including sheep (34) and deer (32, 42). DNA fingerprinting studies suggest that the same clone of E. coli O157:H7 can persist in the same cattle herd; new clones can also be introduced through undetermined routes (12, 21). Incorporation of ceftimoxime and tellurite into media offers improved sensitivity for the recovery of E. coli O157:H7 in bovine feces (46) and may be useful in future surveys.

**CLINICAL MICROBIOLOGY LABORATORY SURVEILLANCE OF E. COLI O157:H7 AND REPORTING REQUIREMENTS**

Unlike most commensal human fecal coliforms, E. coli O157:H7 does not ferment sorbitol after overnight incubation. Therefore, candidate E. coli O157:H7 can be identified on MacConkey agar plates into which sorbitol, rather than lactose, is incorporated as the carbon source. Even though relatively few stools submitted to microbiology laboratories contain E. coli O157:H7, uniform screening and notification policies, and epidemiological and molecular surveillance systems, can be crucial for identifying evolving outbreaks. Failure to detect the early stages of the 1993 outbreak in California (7a) and failure of physicians and laboratorians either to request or to routinely screen for E. coli O157:H7 in Nevada (7b) contributed to the widespread nature of this epidemic.

In the United States, reporting requirements for communicable diseases are set by individual states. At the time of the 1993 epidemic, only 12 states had mandatory reporting laws for E. coli O157:H7 infections. Following the 1993 outbreak, many states evaluated their surveillance systems for foodborne diseases, and by mid-1996, 42 states required the reporting of E. coli O157:H7 infections to public health agencies. However, reporting systems rely on the appropriate detection of infected patients. Despite the ease with which E. coli O157:H7 can be identified by sorbitol-MacConkey agar screening, only about half of randomly surveyed clinical microbiology laboratories in the United States employ this policy (15).

**MOLECULAR FINGERPRINTING OF E. COLI O157:H7**

The routine use of molecular subtyping holds promise as a powerful adjunct to standard epidemiological tools in the investigations of outbreak and sporadic cases of E. coli O157:H7 infection. Because E. coli O157:H7 strains are of clonal descent (54), analysis of certain phenotypes, such as housekeeping enzymes, does not distinguish one strain from another. Other properties, such as antibiograms (33), plasmid profiles (39), and toxin genotype (39, 51), also provide insufficient discriminatory properties to assign strain differences. Therefore, several DNA-based techniques have been used to assess the relatedness of E. coli O157:H7 isolates, including pulse field gel electrophoresis (PFGE) (8) and $\lambda_4$ (25, 44) and Stx gene (stx) (43)-restriction fragment length polymorphism (RFLP) analysis.
PFGE, which distinguishes strains by differences in sizes of large DNA endonuclease digestion fragments, has been used to link outbreak strains of *E. coli* O157:H7 (8) as well as to refute relatedness between strains recovered from patients in the same region and season (4) (Figure 3). PFGE analysis can be applied to a variety of pathogens (53) (i.e., not just *E. coli* O157:H7). However, PFGE cannot always differentiate or link, with certainty, some clones of *E. coli* O157:H7 found in this country (3).

An alternative approach to molecular characterization exploits the polymorphism of λ-bacteriophage sequences surrounding the Stx genes in *E. coli* O157:H7 to differentiate unrelated isolates and to connect epidemiologically linked isolates of *E. coli* O157:H7. Initially, this technique used λ-bacteriophage to probe DNA from *E. coli* O157:H7 digested with a suitable endonuclease (44) (Figure 4). This technique requires electrophoresis and Southern hybridization, and analysis of multiple resulting bands, but λ-RFLP analysis provides a very discriminatory and stable pattern (25, 44). λ-RFLP analysis has linked apparently unconnected cases (33), and random bovine and human isolates in a defined geographic area (41). λ-RFLP analysis has also established that foodborne strains of *E. coli* O157:H7 rapidly disappear from human populations after elimination of the vehicle from the environment, suggesting that humans are not a stable, long-term reservoir for this pathogen, even though person-to-person transmission does occur (25).

Recently, Stx genes have been used to probe *E. coli* O157:H7 restriction fragments. This technique provides discriminatory power equivalent to that of λ-RFLP analysis and is easier to interpret because of the reduced number of bands produced (43) (Figure 5).

**EVOLUTIONARY ASPECTS OF E. COLI O157:H7 PATHOGENICITY**

Recent data suggest that virulence traits and antigens of *E. coli* O157:H7 have been acquired via integration of large segments of chromosomal DNA. Alleles that have been so acquired include stx 1 and 2, which are contained on bacteriophages (50); *eaeA* (28), which encodes intimin, and is encoded on a 35–kilobase pair segment of chromosomal DNA termed the locus of enterocyte effacement (*lee*) region (37); and the *rfb* region of *E. coli* O157:H7, which encodes proteins responsible for the synthesis of the O157 lipopolysaccharide O-side antigen (13). These data suggest that the horizontal transfer of selected DNA fragments, possibly from species other than *E. coli*, may render certain clones of *E. coli* pathogenic.

**STX-PRODUCING E. COLI OTHER THAN E. COLI O157:H7 AS HUMAN PATHOGENS**

Foodborne Stx-producing *E. coli* of serotypes other than *E. coli* O157:H7 are not rare (1, 45), but the frequency with which these non-O157:H7 Stx-producing strains cause human disease in the United States is unknown. Such non-O157:H7 Stx-producing *E. coli* have only rarely been isolated from children in the United States with HUS (49). Non-O157:H7 Stx-producing *E. coli* have also been recovered from sporadic cases of diarrhea (14, 40), but these patients did not develop HUS.

The first and, to date, only, detected outbreak of non-O157:H7 Stx-producing *E. coli* human infections in the United States occurred in Montana (6). Sorbitol nonfermenting *E. coli* O104:H21 were recovered from patients with bloody diarrhea in the Helena area. The implicated vehicle was putatively pasteurized retail milk. These isolates would probably have been overlooked but for the fact that they, like *E. coli* O157:H7, failed to ferment sorbitol after overnight incubation. However, these unusual strains were forwarded to a public health laboratory for serotyping and further analysis. As with the sporadic enteric isolates of non-O157:H7 Stx-producing *E. coli* recovered in Seattle (14), the *E. coli* O104:H21 strains did not cause HUS. However, because the number of non-O157:H7 Stx-producing *E. coli* isolated from humans in the United States has so far been small, the risk of developing HUS in an infected child remains unknown.

Despite concern about the pathogenic potential of non-O157:H7 Stx-producing *E. coli*, *E. coli* O157:H7 remains the predominant precipitant of HUS in the United States.
States. *E. coli* O157:H7 accounted for at least 8 (73%) of 11 cases of HUS in children from western Washington counties admitted to the Children’s Hospital and Medical Center in 1994 and 1995 (unpublished data). This percentage is similar to the percentage (63%) of patients with HUS whose stool cultures yielded this pathogen in our 1985–1987 prospective study (53), in which the yield for children whose stools were cultured before day 7 of illness was 96%, and in the 1993 *E. coli* O157:H7 outbreak, in which the culture positive rate for HUS patients was only 86% (17). These data suggest that *E. coli* of serotype O157:H7 cause almost all cases of postdiarrheal HUS in United States children; failure to recover this pathogen probably reflects delayed or inappropriate stool culture.

Until recently, the identification of non-O157:H7 Stx-producing *E. coli* required the use of cytotoxicity assays or DNA hybridization techniques. Several assays that exploit the binding of Stx to globotriaosylceramide, or enzyme immunoassays, to detect organisms producing this toxin are...
now available (9, 40). This technology should increase the ability of clinical and food microbiologists to identify these potential enteric pathogens. As such technology is disseminated, it will be important to perform studies in human populations that include appropriate control groups to determine which non-O157:H7 Stx-producing E. coli are, indeed, pathogens. It will also remain crucial to isolate the organism(s) producing the Stx, so that research on their ecological niche and study of their virulence traits can be performed.

E. coli O157:H7 remains an important pathogen in the United States. Increased recognition of this organism as a human pathogen by the medical community and general populace, expanded reporting and surveillance systems, and molecular linkage analysis of isolates have contributed to partial control of this problem during the past several years. The pathogenicity of Stx-producing E. coli belonging to serotypes other than O157:H7 requires elucidation.

ACKNOWLEDGMENTS

The authors thank Ms. Christine Merrikin for secretarial assistance and Drs. Marguerite Neill and Mansour Samadpour for helpful and stimulating discussions.

REFERENCES


