

Surveillance for Shiga Toxin-producing *Escherichia coli*, Michigan, 2001–2005

Shannon D. Manning,* Robbie T. Madera,†
William Schneider,† Stephen E. Dietrich,†
Walid Khalife,‡ William Brown,§
Thomas S. Whittam,* Patricia Somsel,†
and James T. Rudrik†

A surveillance system used different detection methods to estimate prevalence of Shiga toxin-producing *Escherichia coli* during 2003–2005 and 2001–2002. More non-O157 serotypes were detected by enzyme immunoassay than by evaluation of non-sorbitol-fermenting *E. coli* isolates. We therefore recommend use of enzyme immunoassay and culture-based methods.

Infection with Shiga toxin-producing *Escherichia coli* (STEC) is a frequent cause of gastrointestinal disease, particularly among children and elderly persons (1). Detection of O157 STEC by culture relies primarily on sorbitol MacConkey agar (SMAC) (2) because O157:H7 strains cannot rapidly ferment sorbitol (3). In contrast, using culture to detect sorbitol-fermenting O157 (4) and non-O157 serotypes is problematic because on SMAC these strains are indistinguishable from other *E. coli*. Consequently, whether the predominance of STEC O157 in disease reflects actual differences in pathogen prevalence or a bias associated with detection is unclear. We therefore sought to determine whether STEC prevalence, particularly of non-O157 serotypes, increased when enhanced detection methods were used.

The Study

The Michigan Department of Community Health implemented a sentinel surveillance system to evaluate blood-containing stool samples from 20 laboratories during April 2003–October 2005 and all stool samples from 2 hospitals during July 2004–October 2005. All suspect non-sorbitol-fermenting *E. coli* from the remaining laboratories were also examined.

*Michigan State University, East Lansing, Michigan, USA;

†Michigan Department of Community Health, Lansing, Michigan, USA;

‡Sparrow Health System, Lansing, Michigan, USA; and

§Detroit Medical Center, Detroit, Michigan, USA

The samples, transported in C&S transport medium (Medical Chemical Corporation, Torrance, CA, USA), were screened for Shiga toxin (Stx) by enzyme immunoassay (EIA) (Meridian BioScience, Cincinnati, OH, USA) after enrichment with gram-negative broth (Remel, Lenexa, KS, USA). EIA is sensitive and specific but cannot detect the Stx2e variant (5), and *Pseudomonas aeruginosa* can produce false-positive results (6). Samples were cultured on SMAC (Remel) and cefixime-tellurite SMAC (7), and samples from the 2 hospitals were tested for occult blood (Beckman Coulter, Fullerton, CA, USA) before EIA testing. Serotyping (Statens Serum Institute, Copenhagen, Denmark; BD Difco, Franklin Lakes, NJ, USA) and real-time PCR for *stx1,2* genes (8) were performed on strains that had positive EIA results, suspect non-sorbitol-fermenting *E. coli*, and multiple colonies of sorbitol-fermenting (SF) strains that had positive EIA results. For some samples, the EIA result was negative but NSF *stx*-positive colonies were detected on SMAC, which indicated a false-negative EIA result. Epidemiologic data were obtained for STEC-positive patients.

During the 5 years studied, 438 STEC were isolated; 401 (92%) were O157. Prevalence over time did not differ ($\chi^2 = 4.14$, degrees of freedom [df] = 4, $p = 0.39$). Similarly, overall prevalence of non-O157 serotypes during 2001–2002 and 2003–2005 did not differ ($\chi^2 = 0.83$, df = 1, $p = 0.36$). Most (70%) STEC isolates were recovered between June and October from heavily populated areas (Figure). No SF O157 were recovered.

In 2001–2002, a total of 664 suspect NSF *E. coli* isolates were evaluated; 179 (27%) were O157 and 2 (0.3%) were non-O157 serotypes (Table 1). After enhanced surveillance began in 2003, a total of 852 suspect isolates were tested; 177 (21%) were O157 and 3 (0.4%) were non-O157 serotypes (Table 1). The remaining STEC ($n = 28$, 2001–2002; $n = 49$, 2003–2005) were detected by EIA. For 5 samples, EIA yielded a false-negative result but STEC

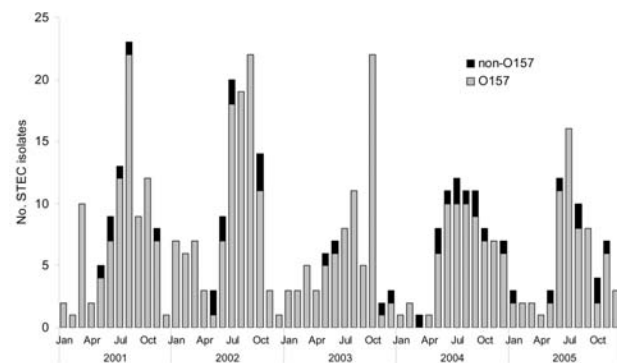


Figure. Frequency of isolation of Shiga toxin-producing *Escherichia coli* (STEC), Michigan, 2001–2005. Enhanced surveillance for STEC began in April 2003.

Table 1. Evaluation of shiga toxin-producing *Escherichia coli* detection methods, Michigan, 2001–2005*

Detection method	Stool samples	Routine STEC surveillance†, no. (%)		Enhanced STEC surveillance, no. (%)		
		2001	2002	2003	2004	2005
Culture and PCR‡ for <i>stx</i> genes of suspect NSF <i>E. coli</i>	Total	327	337	249	317	286
	O157	85 (26)	94 (28)	63 (25)	58 (18)	56 (20)
	Non-O157	2 (0.6)	0 (0)	0 (0)	2 (0.6)	1 (0.3)
EIA of bloody or suspect samples	Total	N/A	N/A	209	141	73
	O157	4	11	11 (5)	7 (5)	6 (8)
	Non-O157	4	9	4 (2)	7 (5)	7 (10)
EIA of all samples	Total	N/A	N/A	N/A	1,405	1,021
	O157	N/A	N/A	N/A	5 (0.4)	1 (0.1)
	Non-O157	N/A	N/A	N/A	1 (0.07)	0 (0)

*STEC, Shiga toxin-producing *Escherichia coli*; NSF, non-sorbitol-fermenting; EIA, enzyme immunoassay.

†In 2001 and 2002, EIA of bloody stools was performed by 2 large clinical laboratories in Michigan and was not part of the enhanced STEC surveillance system at the Michigan Department of Community Health. Therefore, the total number of EIAs performed is not known; the total number of EIA s submitted as positive for Stx is provided by serotype.

‡PCR for *stx*1,2 genes was not initiated at the Michigan Department of Community Health until 2002. Before 2002, a DNA probe (Digene Signal Kit, Digene Diagnostic, Inc., Silver Spring, MD, USA) was used to detect STEC.

were recovered from SMAC. During 2003–2005, 7 of the 49 STEC came from screening all 2,426 samples from the 2 hospitals; 359 (15%) of these samples contained occult blood. Among the 7 detected, 5 contained occult blood and 6 were O157. The remaining 42 (10%) STEC were found by screening 423 suspect samples from other laboratories; 18 (4%) were non-O157 serotypes. More non-O157 serotypes were detected ($\chi^2 = 61.1$, $df = 1$, $p < 0.00001$) from 2003–2005 when EIA was used instead of the NSF *E. coli* isolate submission process. Among all 37 non-O157 serotypes isolated, O45:H2 ($n = 10$) and O26:H11 ($n = 5$) predominated.

Epidemiologic data were available for up to 389 (89%) STEC patients, depending on the variable assessed. Most patients (62%) were Caucasian; $\approx 50\%$ were female. Disease occurred mostly in persons ≤ 10 (27%), 11–18 (19%), and 19–30 (17%) years of age. Although disease frequency was lower (9%) in persons > 65 years of age, these persons were more likely to be hospitalized than were persons ≤ 18 years of age, as were persons with bloody diarrhea or hemolytic-uremic syndrome (HUS) (Table 2). Among the 12 patients with HUS, 2 were infected with non-O157 serotypes O103:H2 and O76:H7, and 7 of the 12 HUS-associated strains were *stx*2 only.

To adjust for factors associated with hospitalization, we fit a logistic regression model that included age and symptom variables in the model. The adjusted associations were similar to the crude associations. Hospitalization was more frequent for persons with bloody diarrhea (adjusted odds ratio [OR] 1.8, 95% confidence interval [CI] 1.04–3.08) and HUS (adjusted OR 16.0, 95% CI 2.00–127.47). Also, persons 19–64 (adjusted OR 1.6, 95% CI 1.05–2.59) and > 65 (adjusted OR 6.6, 95% CI 2.57–17.15) years of age were hospitalized more frequently than persons ≤ 18 years of age.

Conclusions

Enhanced detection methods did not significantly increase the year-to-year recovery of STEC. Overall, the observed STEC prevalence decreased slightly over time, similar to the national trend of an overall 42% decrease in STEC O157 incidence during 1996–2004 (9). This reduction is likely attributable to numerous factors, including heightened consumer awareness (9) and improved screening protocols during food production (10).

Enhanced surveillance did, however, enhance detection of non-O157 serotypes; 4.3% of EIA-positive stools were non-O157 compared with 0.5% of suspect NSF *E. coli*. Additionally, among the STEC found, 34 (48%) were non-O157 and 37 (52%) were O157 when EIA was used on suspect stools, compared with only 3 (1.6%) non-O157 and 177 (98.3%) O157 among NSF *E. coli*. Despite enhanced surveillance, STEC prevalence is probably still underestimated, particularly for non-O157 serotypes, because not all ill persons seek medical care and not all laboratories submit suspect stools for evaluation. Nevertheless, in 5 years, our surveillance identified 66 (15%) cases that would have been undetected by conventional methods; 31 (47%) were non-O157. Among those patients for whom data were available, 27 (42%) of 64 were < 18 years of age, 22 (43%) of 51 were hospitalized, and 39 (76%) of 51 had bloody diarrhea. Although bloody stool and patient age are poor predictors of STEC infection (11), our analysis demonstrates that screening bloody stool samples improves detection of non-O157, and blood and older age are important predictors of more severe disease, which may be more costly if undetected.

Hospitalization of STEC patients with and without HUS costs an estimated US \$30,307 and \$4,061 per patient, respectively (12). Therefore, Michigan hospital costs associated with STEC infection likely exceeded

Table 2. Association of characteristics with infection by Shiga toxin–producing *Escherichia coli* among 389 of 438 patients for whom data were available, Michigan, 2001–2005*†

Characteristic	No. with characteristic	No. (%) hospitalized	OR (95% CI)
Demographics			
Age, y			
≤18	178	77 (43)	1.0
19–64	178	94 (53)	1.5 (0.95–2.28)
≥65	32	26 (81)	5.7 (2.09–16.27)
Sex			
Female	204	105 (51)	1.0
Male	185	92 (50)	0.9 (0.61–1.42)
Clinical symptoms			
Abdominal pain			
No	76	40 (53)	1.0
Yes	304	151 (50)	0.9 (0.54–1.47)
Body aches			
No	323	161 (50)	1.0
Yes	57	30 (53)	1.1 (0.64–1.96)
Chills			
No	313	155 (50)	1.0
Yes	66	36 (55)	1.2 (0.72–2.08)
Diarrhea			
No	154	82 (53)	1.0
Yes	226	109 (48)	0.8 (0.54–1.23)
Bloody diarrhea			
No	77	30 (39)	1.0
Yes	303	161 (53)	1.8 (1.10–2.96)
HUS			
No	368	180 (49)	1.0
Yes	12	11 (92)	11.5 (1.47–89.90)
Bacterial serotype and genes			
O157	360	181 (50)	1.0
Non-O157	29	16 (55)	1.2 (0.57–2.60)
<i>stx1</i>	28	14 (50)	1.0
<i>stx2</i>	121	54 (45)	0.8 (0.33–1.98)
<i>stx1,2</i>	239	128 (54)	1.2 (0.49–2.70)

*OR, odds ratio; CI, confidence interval; HUS, hemolytic-uremic syndrome.

†Some numbers do not add up to the total (n = 389) because of missing values (never >2.6%).

\$1,119,050 during 2001–2005, as 198 patients were hospitalized and 12 had HUS. Identification of each additional STEC case could have a substantial public health effect in that 1 case may lead to the recognition of an outbreak, which if detected early, could contribute to a cost savings as well as reduced STEC-associated illness. We estimated that the cost to detect each of the 66 additional cases using the EIA (\$7 per test including labor) differed considerably when we evaluated screening of all stool samples (\$2,426/per positive) versus suspect stool samples (\$10/per positive).

No widely available test detects all STEC, and use of multiple methods is not cost-effective. Consequently, we recommend using EIA in conjunction with SMAC culture to recover isolates for molecular characterization and subsequent outbreak investigations. Although occult blood tests did not enhance the sensitivity of STEC recovery, patient data and accompanying epidemiologic information may help identify which samples to test, thereby prevent-

ing future outbreaks. Because such epidemiologic information is often not available to laboratory personnel, we suggest that clinical laboratories work with medical administrations to use EIAs as their standard of practice and to facilitate routine availability of such information. Until more sensitive and cost-effective STEC screening methods are available, facilities that cannot implement EIAs should forward stool samples that are suspect, as well as those with positive screening results, to public health laboratories. These laboratories can easily evaluate suspect stools for STEC by EIA or PCR followed by culture of all positive samples to recover the isolate for further characterization.

Acknowledgments

We thank Francis Pouch Downes, Carrie Anglewicz, Barbara Evans, Kendra Anspaugh, Kelly Scott, Hao Trinh, Ben Hutton, Jon George, John Dyke, Kevin Rodeman, Jeff Massey, Steve Tharpe, Mary Grace Stobierski, Jim Collins, Michael Hass,

Sally Bidol, Theresa Painter, James Wolcott, Eileen Matthews, Janet Green, Dave Aldrich, Carl Pierson, Duane Newton, Cora Manby, Richard Van Enk, Alan Petkus, Joan Baughman, Linda Hayka, Vito Damore, Cindy Miilu, Sharon Gauthier, John Kuehn, Marie Graham, Therese Carson, and Daniel Sundin.

The Emerging Infectious Diseases Research Fellowship Program, administered by the Association of Public Health Laboratories and funded by the US Centers for Disease Control and Prevention (CDC), supported S. Manning's appointment (2002–2004), and the CDC/Council of State and Territorial Epidemiologists Applied Epidemiology Fellowship Program supported R. Madera's appointment (2003–2005). This project was supported in part with federal funds for the Food and Waterborne Disease Integrated Research Network under National Institutes of Health research contract no. N01-AI-30058.

Dr Manning is a molecular and infectious disease epidemiologist. She studies the molecular epidemiology of pathogenic *E. coli* and group B *Streptococcus* at Michigan State University.

References

1. Thorpe CM. Shiga toxin-producing *Escherichia coli* infection. *Clin Infect Dis*. 2004;38:1298–303.
2. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol*. 1986;23:869–72.
3. Farmer JJ, Davis BR. H7 antiserum-sorbitol fermentation medium: a single tube screening medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol*. 1985;22:620–5.
4. Karch H, Bielaszewska M. Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H(-) strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. *J Clin Microbiol*. 2001;39:2043–9.
5. Allerberger F, Rossboth D, Dierich MP, Aleksic S, Schmidt H, Karch H. Prevalence and clinical manifestations of Shiga toxin-producing *Escherichia coli* infections in Austrian children. *Eur J Clin Microbiol Infect Dis*. 1996;15:545–50.
6. Beutin L, Zimmermann S, Gleier K. *Pseudomonas aeruginosa* can cause false-positive identification of verotoxin (Shiga-like toxin) production by a commercial enzyme immune assay system for the detection of Shiga-like toxins (SLTs). *Infection*. 1996;24:267–8.
7. Zadik PM, Chapman PA, Siddons CA. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. *J Med Microbiol*. 1993;39:155–8.
8. Reischl U, Youssef MT, Kilwinski J, Lehn N, Zhang WL, Karch H, et al. Real-time fluorescence PCR assays for detection and characterization of Shiga toxin, intimin, and enterohemolysin genes from Shiga toxin-producing *Escherichia coli*. *J Clin Microbiol*. 2002;40:2555–65.
9. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 sites, United States, 2004. *MMWR Morb Mortal Wkly Rep*. 2005;54:352–6.
10. Naugle AL, Holt KG, Levine P, Eckel R. Food safety and inspection service regulatory testing program for *Escherichia coli* O157:H7 in raw ground beef. *J Food Prot*. 2005;68:462–8.
11. Kehl KS, Havens P, Behnke CE, Acheson DW. Evaluation of the premier EHEC assay for detection of Shiga toxin-producing *Escherichia coli*. *J Clin Microbiol*. 1997;35:2051–4.
12. Frenzen PD, Drake A, Angulo FJ. Economic cost of illness due to *Escherichia coli* O157 infections in the United States. *J Food Prot*. 2005;68:2623–30.

Address for correspondence: James T. Rudrik, Michigan Department of Community Health, Bureau of Laboratories, 3350 North Martin Luther King Jr Blvd, Bldg 44, Rm 129, Lansing, MI 48909, USA; email: RudrikJ@michigan.gov

Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

The Public Health Image Library (PHIL)



The Public Health Image Library (PHIL), Centers for Disease Control and Prevention, contains thousands of public health-related images, including high-resolution (print quality) photographs, illustrations, and videos.

PHIL collections illustrate current events and articles, supply visual content for health promotion brochures, document the effects of disease, and enhance instructional media.

PHIL Images, accessible to PC and Macintosh users, are in the public domain and available without charge.

Visit PHIL at <http://phil.cdc.gov/phil>.

Search past issues of EID at www.cdc.gov/eid