MAJOR ARTICLE

Characteristics of O157 versus Non-O157 Shiga Toxin–Producing *Escherichia coli* Infections in Minnesota, 2000–2006

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Background. *Escherichia coli* O157:H7 (O157) is the Shiga toxin–producing *E. coli* (STEC) serotype most frequently isolated and most often associated with hemolytic uremic syndrome (HUS) in the United States. Non-O157 STEC serotypes can also cause serious illness, but their impact as pathogens remains undefined. We compared characteristics of non-O157 and O157 STEC infections identified through sentinel surveillance.

Methods. Sentinel sites included a metropolitan health maintenance organization laboratory and a hospital laboratory serving a small city and rural area. We received sorbitol-MacConkey agar plates from every stool culture performed at both sites during 2000–2006. Colony sweeps were screened for *stx1* and *stx2* by polymerase chain reaction. *E. coli* identity, serotype, and presence of *stx1* and/or *stx2* were confirmed on individual isolates.

Results. Two hundred six STEC isolates were identified: 108 (52%) were non-O157 serotypes, and 98 (48%) were O157. Of non-O157 cases, 54% involved bloody diarrhea, and 8% involved hospitalization. Non-O157 isolates with at least *stx2* were not more likely to cause severe illness (bloody diarrhea, hospitalization, or HUS) than were non-O157 isolates with only *stx1*. O157 cases were more likely than non-O157 cases to involve bloody diarrhea (78% vs 54%; $P < .001$), hospitalization (34% vs 8%; $P < .001$), and HUS (7% vs 0%; $P = .005$). When including only isolates with at least *stx2*, O157 cases were still more likely to involve bloody diarrhea (78% vs 56%; $P = .02$) and hospitalization (33% vs 12%; $P = .01$) than non-O157 cases.

Conclusions. Differences in severity among STEC infections could not be explained by *stx2*, suggesting that additional factors are important in STEC virulence.

Shiga toxin–producing *Escherichia coli* (STEC) is a group of pathogenic *E. coli* that cause diarrhea, bloody diarrhea, and hemorrhagic colitis. Hemolytic uremic syndrome (HUS) develops in 5%–10% of individuals with STEC-associated diarrhea and has a case-fatality rate of 3%–7% [1].

Cattle are a reservoir of STEC, and foods originating from or contaminated by cattle are important vehicles for human disease. Other routes of infection include consumption of contaminated water, direct contact with infected ruminant animals, and person-to-person transmission [1–3].

More than 200 *E. coli* serotypes produce Shiga toxin, and >100 of these have been linked with human illness [1]. *E. coli* O157 (O157) is the serotype most frequently isolated and most often associated with HUS in the United States. Non-O157 STEC (non-O157) serotypes can also cause serious illness and have been implicated in outbreaks in the United States and elsewhere [4–8].

Variability exists between non-O157 STEC serotypes in their associations with outbreaks and disease severity. Differences in virulence factors produced by different strains likely help explain this variability [9, 10]. Shiga toxin has been considered the primary virulence factor responsible for causing severe illness, including bloody diarrhea and HUS, but not all STEC infections lead to these conditions. This suggests that other determinants, in addition to host factors, are involved [9]. Shiga toxins are classified into 2 main categories—Shiga toxin

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1 (Stx1) and Shiga toxin 2 (Stx2)—and each group contains variants. Previous studies have found that STEC strains producing Stx2 are more likely to cause HUS than are strains that produce Stx1 alone [11–13].

Detection of O157 in stool specimens relies mainly on its inability to rapidly ferment sorbitol; therefore, sorbitol-MacConkey (SMAC) agar is used to culture O157. However, most non-O157 STEC do ferment sorbitol and cannot be differentiated from non-pathogenic *E. coli* strains on SMAC agar. Therefore, detection of non-O157 STEC involves either testing directly for Shiga toxins or the genes that encode them [1, 3].

Because non-O157 STEC are not routinely detected through standard stool culture methods in clinical laboratories, their impact as pathogens remains largely undefined. We used sentinel surveillance in Minnesota to determine the burden of non-O157 infections relative to O157, and to compare clinical and epidemiological characteristics of O157 and non-O157 STEC infections identified through the sentinel sites.

METHODS

Sentinel surveillance sites. Data were collected through 2 sentinel surveillance sites in Minnesota during 2000–2006. One was the laboratory for a large Minneapolis-St. Paul metropolitan area health maintenance organization; the other was a hospital laboratory that serves a small city and surrounding rural area that is rich in food animal agriculture, particularly dairy production. The estimated maximum population sizes served by these systems were 656,000 (urban site) and 643,000 (rural site). All stool samples submitted to the 2 laboratories for enteric bacterial culture were plated on SMAC enteric stool culture plates; after completion of testing, the SMAC plates were sent to the Minnesota Department of Health Public Health Laboratory (St. Paul).

Laboratory methods. Upon receiving a SMAC plate from a sentinel laboratory, template DNA was prepared from colony sweeps. Six sweeps were made through representative areas of growth using a 1.0-μL disposable loop. Sweeps included all visible colony morphologies and avoided the primary inoculation area. Individual sweeps from each primary specimen were mixed. One loopful of the mixed sweep material and 200 μL of molecular grade water (Sigma) were heated for 15 min in boiling water and centrifuged at 16,000 *g* for 2 min. Clear supernatants containing bacterial DNA were withdrawn for polymerase chain reaction analysis.

From January 2000 through July 2005, Shiga toxin genes *stx1* and *stx2* were detected using previously described primers and amplification methods [14]. In July 2005, a different method [15] was implemented to increase Shiga toxin gene detection sensitivity.

Up to 24 individual colonies from samples that initially tested positive for *stx1* or *stx2* were retested by polymerase chain reaction. Shiga toxin gene-positive isolates were identified by standard biochemical methods [16]. Somatic and flagellar antigens were determined using Denka Seiken antisera. Isolate identity was further confirmed at the Centers for Disease Control and Prevention (Atlanta, GA). If no individual colony was found to contain Shiga toxin gene sequences, the sample was classified as a polymerase chain reaction–positive STEC of unknown serotype. If an individual *E. coli* colony was Shiga toxin gene–positive and tested negative for O157, it was classified as a non-O157 STEC even if the serogroup could not be determined.

Data collection. If STEC was detected, the submitting clinic provided case demographic and clinical information. Interviews were attempted for all STEC cases to collect additional demographic, symptom, exposure, and treatment information. Similar demographic and treatment information was collected for HUS cases reported to MDH. Only patients with HUS for whom STEC was confirmed through the sentinel surveillance during 2000–2006 were included in this investigation.

Statistical analyses. Comparisons were made between non-O157 and O157 STEC cases in Minnesota residents at the 2 sentinel sites. Cases were excluded if the serogroup of the identified STEC was undetermined, unless an *E. coli* isolate was Shiga toxin gene positive and tested negative for O157; in that instance, the corresponding case was classified as a non-O157 STEC and included. Cases for which samples yielded another gastrointestinal pathogen were excluded because of the potential impact on illness severity. Secondary and outbreak-associated cases were included for clinical comparisons but excluded for risk factor analyses.

Results were obtained using SAS statistical software, version 9.1 (SAS Institute). The χ^2 test for linear trend was used to analyze Shiga toxin type changes over time. Categorical variables were analyzed using Fisher's exact test. The Kruskal-Wallis test was used to compare continuous variables. Multivariate analysis using logistic regression was performed following a stepwise selection process. Two-sided *P* values $\leqslant$.05 were considered to be statistically significant.

RESULTS

There were 302 Shiga toxin gene–positive SMAC plates identified from the 28,380 plates tested from the sentinel sites during 2000–2006 (median number of plates tested per year, 3984; range, 3612–4842). Of these, Shiga toxin–producing *E. coli* colonies were isolated for 226 (75%); therefore, for the other 76 (25%), the serogroup was unknown. Among the 226 isolated STEC, non-O157 serogroups accounted for 119 (53%), O157 accounted for 100 (44%), and the serogroup was defined as "rough" for 7 (3%).

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The number and proportion of STEC cases identified each year displayed a downward trend during 2000–2004, but then increased from 2004–2006 (2000, 53 cases [1.2% of plates submitted]; 2001, 53 cases [1.3%]; 2002, 36 cases [0.9%]; 2003, 25 cases [0.7%]; 2004, 22 cases [0.6%]; 2005, 50 cases [1.3%]; and 2006, 63 cases [1.3%]). Most STEC samples were collected during July-October (183 samples [61%]). Both the O157 and non-O157 serogroups exhibited a similar summer/early fall seasonality (figure 1).

Twenty-two (7%) of the 302 patients with STEC were infected with another gastrointestinal pathogen and were excluded from further analyses. Of these, 8 had enterotoxigenic *E. coli* infection, 7 had *Campylobacter* infection, 3 had *Cryptosporidium* infection, 3 had *Giardia* infection, and 1 had *Salmonella* infection. Remaining isolates for which the serogroup was unknown or rough ($n = 74$) were also excluded.

Of the remaining 206 STEC cases, 108 (52%) involved non-O157 serotypes, and 98 (48%) involved O157. The urban site yielded 28 O157 and 54 non-O157 STEC isolates, whereas the rural site yielded 70 O157 and 54 non-O157 STEC isolates.

Five serogroups represented 74% of the 108 non-O157 isolates: O26 (29 isolates [27%]), O103 (23 [21%]), O111 (20 [19%]), O145 (5 [5%]), and O45 (3 [4%]). The remaining serogroups included O1, O21, O22, O51, O71, O76, O80, O88, O91, O119, O121, O128, O137, O159, O165, O166, and O175. The serogroup could not be determined for 6 non-O157 isolates (6%).

STEC case demographic characteristics. Interviews were conducted for ~90% of patients with non-O157 and O157 infection. Individuals infected with non-O157 and O157 STEC were similar with regard to age, sex, race, ethnicity, and antibiotic treatment status (defined as treatment with any antibiotic). The median age for all patients with STEC infection was 15 years (range, 15 days to 88 years), and 118 (57%) individuals were female. Race information was available for 186 patients (90%); 177 (95%) were white, 6 (3%) were black, 2 (1%) were Asian/Pacific Islander, and 1 (1%) reported "other." Ethnicity was reported for 182 patients (88%); 177 (97%) were non-Hispanic. Time from illness onset to specimen collection and from onset to interview was significantly longer for patients with non-O157 cases than for those with O157 cases (median, 4 vs 3 days [$P = .006$] and 45 vs 14 days [$P < .001$], respectively).

Risk factor analysis. In the 7 days before illness onset, there was no significant difference between O157 and non-O157 groups with regard to the proportion of patients who consumed unpasteurized milk; drank untreated or "raw" water; reported swimming in a lake, river, ocean, or pool; consumed meat from a place other than a grocery store (eg, a butcher shop or by private slaughter); lived on a farm; visited a farm or petting zoo; or ate at a restaurant (table 1). Patients with non-O157 infection were more likely to have travelled internationally (12% vs 1%; $P = .01$), and those with O157 infection were more likely to have consumed ground beef (76% vs 58%; $P = .03$) (table 1). In the rural population, patients with O157 and non-O157 infection were similar with regard to all exposures (data not shown). In the urban population, patients with non-O157 infection were more likely to have consumed raw water than were those with O157 infection (24% vs 0%; $P = .02$) before illness onset.

Shiga toxin type. Among the 108 non-O157 STEC isolates, 65 (60%) had *stx1* alone, 14 (13%) had *stx2* alone, and 29 (27%) had both toxin genes; therefore, 43 non-O157 isolates (40%) had at least *stx2*. The proportion of non-O157 STEC samples that contained only *stx1* increased significantly from 2000 to 2006, whereas the proportion that had both toxins decreased significantly (figure 2). Among the 98 O157 isolates, 1 (1%) had *stx1* alone, 30 (31%) had *stx2* alone, and 67 (68%) produced both toxins. There was no significant change in Shiga toxin gene type since 2000 for O157 isolates (figure 2). Non-

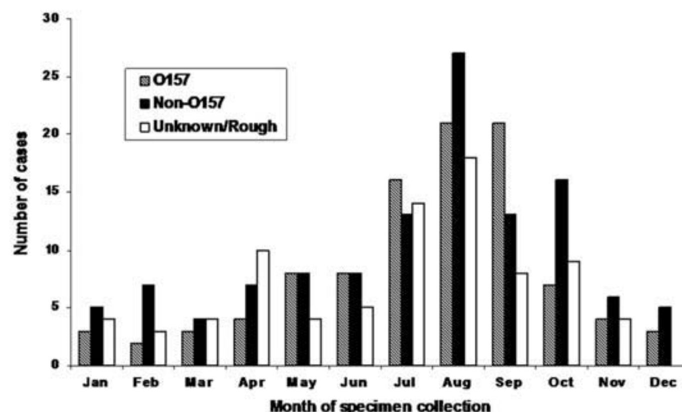

Figure 1. Number of Shiga toxin–producing *Escherichia coli* cases identified through sentinel surveillance in Minnesota, by serogroup and month of specimen collection, 2000–2006.

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Table 1. Comparison of Potential Exposures for O157 and Non-O157 Shiga Toxin-Producing *Escherichia coli* Cases Identified in Minnesota through Sentinel Surveillance, 2000–2006.

	No. (%) of patients[a]			
Exposure	O157 (n = 76)	Non-O157 (n = 94)	OR (95% CI)	P
International travel	1 (1)	11 (12)[b]	0.1 (0.01–0.8)	.01
Consumption of unpasteurized milk	1 (1)	2 (2)	0.6 (0.05–6.9)	>.99
Consumption of raw water	13 (22)	28 (31)	0.6 (0.3–1.2)	.2
Swimming	18 (25)	31 (36)	0.6 (0.3–1.2)	.2
Consumption of meat from a butcher or private slaughter	20 (27)	20 (24)	1.2 (0.6–2.5)	.7
Lives on a farm	10 (14)	8 (9)	1.7 (0.6–4.4)	.3
Visit to a farm/petting zoo	14 (20)	14 (16)	1.3 (0.6–3.0)	.5
Household pet contact	47 (68)	55 (67)	1.0 (0.5–2.1)	>.99
Daycare exposure	18 (24)	17 (21)	1.2 (0.6–2.6)	.7
Restaurant exposure	55 (79)	56 (71)	1.5 (0.7–3.2)	.3
Consumption of ground beef	53 (76)	42 (58)	2.2 (1.1–4.6)	.03

[a] Responses were not available for all cases for some variables.
[b] Countries of travel were Mexico, 6 patients; and Egypt, Guatemala, Italy, Italy and Croatia, and Morocco and Spain, 1 patient each.

O157 isolates were more likely to have *stx1* alone than were O157 isolates (65 [60%] vs 1 [1%]; $P < .001$), whereas O157 isolates were more likely to have *stx2* alone (30 [31%] vs. 4 [13%]; $P = .03$) or both toxins (65 [66%] vs 29 [27%]; $P < .001$).

Clinical comparison. Patients infected with any STEC (i.e., O157 and non-O157 combined) that had at least *stx2* were more likely to develop bloody diarrhea (71% vs 53%; $P = .03$) and to be hospitalized (27% vs 8%; $P = .001$) than were those infected with any STEC that had *stx1* alone, regardless of serogroup (table 2). However, for non-O157 STEC only, there were no significant differences in illness severity between cases involving isolates that had at least *stx2* and those that had only *stx1* (table 2). Patients infected with any STEC that had *stx2* alone were not significantly different from patients infected with STEC that had *stx1*, alone or in combination with *stx2*, with regard to severity of illness (data not shown).

Cases with O157 infection were more likely to involve bloody diarrhea (78% vs 54%; $P < .001$), hospitalization (34% vs 8%; $P < .001$), and HUS (7% vs 0%; $P = .005$), compared with cases of non-O157 infection (table 3). Five of the patients with HUS were 1–3 years old, 1 was 20 years old, and 1 was 87 years old. Patients with O157 infection reported having a greater maximum number of stools per 24-h period than did those with non-O157 infection (median, 15 vs 10; $P < .001$). Patients with non-O157 infection reported a longer duration of diarrhea than did those with O157 infection (median, 7 vs 6 days; $P = .004$) (table 3). However, only 56 patients with O157 infection

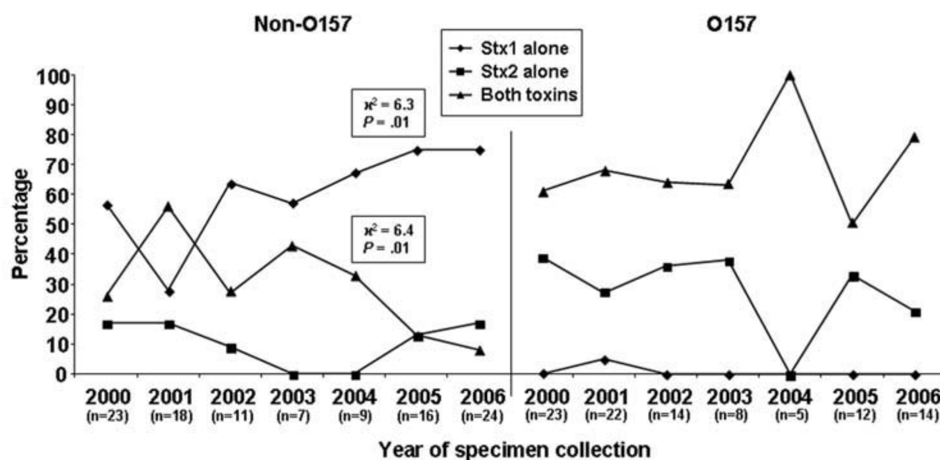

Figure 2. Type of Shiga toxin produced by O157 and non-O157 Shiga toxin–producing *Escherichia coli* isolates, by year of specimen collection, 2000–2006. Stx1, Shiga toxin 1; Stx2, Shiga toxin 2.

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Table 2. Clinical Outcomes in Shiga Toxin-Producing *Escherichia coli* Cases Identified in Minnesota through Sentinel Surveillance by Shiga Toxin Gene Type, 2000–2006.

Variable	Cases involving O157 and non-O157 combined			Only non-O157 cases		
	At least *stx2* ($n = 140$)	*stx1* alone ($n = 66$)	*P*	At least *stx2* ($n = 43$)	*stx1* alone ($n = 65$)	*P*
Diarrhea	124 (98)	58 (98)	>.99	38 (97)	57 (98)	>.99
Fever	41 (33)	25 (45)	.2	13 (33)	24 (44)	.4
Bloody diarrhea	89 (71)	31 (53)	.03	22 (56)	30 (53)	.8
Hospitalization	37 (27)	5 (8)	.001	5 (12)	4 (6)	.3
Hemolytic uremic syndrome	7 (5)	0 (0)	.1	0	0	
Diarrhea duration, median days	7	6	.01	7	7	.4
Median maximum no. of stools per 24-h period	8	11	.01	10	10	.4

NOTE. Data are no. (%) of cases, unless noted otherwise. Responses were not available for all cases for some variables.

(57%) had recovered when interviewed, compared with 81 (75%) of those with non-O157 infection.

When we restricted the analysis to include only STEC isolates that had *stx2*, either alone or with *stx1* ($n = 140$), patients with O157 infection were still significantly more likely to develop bloody diarrhea (78% vs 56%; $P = .02$) and to be hospitalized (33% vs 12%; $P = .01$) than were those with non-O157 infection (table 3). Patients with O157 infection also reported having a greater maximum number of stools per 24-h period (median, 15 vs 10; $P = .002$) (table 3). When we included only isolates that had *stx2* alone, patients with O157 infection ($n = 30$) were significantly more likely than those with non-O157 infection ($n = 14$) to develop bloody diarrhea (83% vs 42%; $P = .02$).

Demographic, serogroup, and Shiga toxin type were analyzed using logistic regression for the outcomes of bloody diarrhea and HUS. No factors were statistically associated with HUS in the multivariate analysis. The O157 serogroup was the only variable associated with bloody diarrhea (adjusted odds ratio, 3.1; 95% confidence interval, 1.5–6.4; $P = .003$).

DISCUSSION

Non-O157 STEC isolates were recovered from stool specimens obtained from ill patients slightly more frequently than O157 when results from the sentinel sites were combined. This suggests that non-O157 serotypes account for a substantial proportion of STEC infections in Minnesota and is consistent with other studies in the United States on the relative incidence of O157 versus non-O157 [17–21]. Three serogroups (O26, O103, and O111) accounted for 67% of the non-O157 isolates of known serogroup in this study. These serogroups are among the 6 most common serogroups in the United States [12]. Most non-O157 cases in Minnesota were identified during the summer months, following a seasonal trend consistent with that of O157 [12].

It was hypothesized that individuals in rural settings would be more likely to contact farm animals and settings than urban populations and, therefore, that STEC infection would occur more frequently in the rural population. The rural population

Table 3. Clinical Outcomes in Shiga Toxin-Producing *Escherichia coli* Cases Identified in Minnesota through Sentinel Surveillance by Serogroup, 2000–2006.

Variable	All cases			Only cases with at least *stx2*		
	O157 ($n = 98$)	Non-O157 ($n = 108$)	*P*	O157 ($n = 97$)	Non-O157 ($n = 43$)	*P*
Diarrhea	87 (99)	95 (98)	>.99	86 (99)	38 (97)	.5
Fever	29 (34)	37 (39)	.5	28 (33)	13 (33)	>.99
Bloody diarrhea	68 (78)	52 (54)	<.001	67 (78)	22 (56)	.02
Hospitalization	33 (34)	9 (8)	<.001	32 (33)	5 (12)	.01
Hemolytic uremic syndrome	7 (7)	0 (0)	.005	7 (7)	0 (0)	.1
Diarrhea duration, median days	6	7	.004	6	7	.1
Mean maximum no. of stools per 24-h period	15	10	<.001	15	10	.002

NOTE. Data are no. (%) of cases unless noted otherwise. Responses were not available for all cases for some variables.

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accounted for 63% of STEC cases in this study. Non-O157 cases were identified with the same frequency in the urban and rural populations. However, the distribution of O157 cases differed markedly by site. The rural site accounted for 71% of all O157 cases in the study. O157 cases occurred more frequently than non-O157 cases in the rural population but were far less common than non-O157 cases in the urban population. This suggests that farm-related factors may be more important for O157 than non-O157 STEC. However, our risk factor analysis did not provide additional evidence for that. In fact, patients with non-O157 infection were similar to those with O157 infection with regard to exposure to several established O157 risk factors, including living on or visiting a farm, consumption of unpasteurized milk, and procuring meat through private slaughter [22, 23]. Non-O157 cases were significantly more likely to have travelled internationally. When stratifying the population by sentinel site, patients with non-O157 STEC infection from the urban site were significantly more likely to have consumed raw water. Conversely, a significantly higher proportion of patients with O157 reported consuming ground beef. These results suggest that whereas some risk factors for O157 and non-O157 STEC infection are the same, the epidemiology of these 2 groups may differ in important ways. Future studies to evaluate risk factors for non-O157 STEC infection independently are necessary.

Non-O157 infections caused substantial morbidity in our study cases; 54% of patients reported bloody diarrhea, 8% were hospitalized, and the median duration of diarrhea was 7 days. However, patients with O157 infection were significantly more likely to develop severe illness, as measured by bloody diarrhea, HUS, hospitalization, and maximum number of stools per 24-h period. To assess the impact of *stx2* on the difference in illness severity between O157 and non-O157 STEC, stratified analyses were performed using only isolates that had *stx2,* either in combination with *stx1* or alone. Patients infected with *stx2*-containing O157 STEC were still more likely to develop bloody diarrhea and be hospitalized than were those who were infected with *stx2*-containing non-O157 STEC. If *stx2* is the primary STEC virulence determinant, as has been proposed, these 2 STEC categories should have been similar with regard to illness severity after controlling for this factor. Furthermore, there were no differences when we compared patients infected with non-O157 serotypes that had only *stx1* with those for which the serotypes had at least *stx2.* If toxin type is a stronger determinant of severe illness than serogroup, differences should have been seen within non-O157 cases by toxin type as well.

In our investigation, *stx1* and *stx2* were the only virulence factors evaluated. However, other genes or gene variants have been proposed to be associated with severe clinical outcomes [9, 24]. Among these is the hypothesis that certain Shiga toxin variants are more likely to cause severe disease (ie, the specific variant rather than the broader toxin group may have a greater influence on the course of illness) [24–27]. Subtyping of Shiga toxin gene variants to assess the association between toxin subtypes and clinical outcomes is warranted, particularly for *stx2.* In addition, the influence of other putative virulence factors on the pathogenicity of STEC infections, including the additive effects of these factors, should be evaluated [27].

This study had some potentially important limitations. Because of detection methods, patients with O157 infection were interviewed significantly closer to onset than were those with non-O157 infection. Poor recall or recall bias may have been introduced in the interviews with non-O157–infected patients as a result of the delay, causing patients to inaccurately report illness details and/or exposure histories. Therefore, some of the differences observed between the non-O157 and O157 groups may have been influenced by differences in recall. Another limitation was the change in STEC laboratory detection methods at the Minnesota Department of Health Public Health Laboratory that occurred during 2005. The new STEC detection method likely had an increased sensitivity for *stx1*. Therefore, the increase in non-O157 STEC found to contain *stx1* in the last 2 years of the study may have been influenced by enhanced testing methods and not represent real trends.

The results of this study have clinical implications. Testing of samples to identify the presence of *stx2* versus *stx1* may not be reliable in predicting whether the infecting STEC strain is capable of causing severe illness. Serogroup (O157 vs non-O157) was an important predictor of severity, but it is apparent that more-specific testing (ie, for *stx2* subtypes or other virulence factors) is necessary to identify the specific virulence factors that contribute to severe disease.

In summary, non-O157 STEC infections were slightly more common than O157 infections in our study. Overall, O157 infections were more severe, but non-O157 infections caused substantial morbidity as well. Improvements in diagnostic capabilities will lead to increased detection of non-O157 STEC cases. This should lead to the identification of additional outbreaks and risk factors for this group of organisms. It also should result in a better understanding of the determinants associated with severe disease caused by STEC.

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