Shiga Toxin-Producing *Escherichia coli* in Diarrheal Stool of Swedish Children: Evaluation of Polymerase Chain Reaction Screening and Duration of Shiga Toxin Shedding

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Received November 13, 2014; accepted January 13, 2015; electronically published February 17, 2015.

Background. Shiga toxin (Stx)-producing *Escherichia coli* (STECs) are the most common cause of acute renal failure in children. The present study evaluated a 10-year STEC polymerase chain reaction screening regimen in children.
Methods. All routine stool culture specimens from patients below 10 years of age (n = 10 342) from May 2003 through April 2013 in the County of Jönköping, Sweden, were included. Patients were divided in 1 group where analyses of STEC were requested by the clinician (n = 2366) and 1 screening group (n = 7976). Patients who were positive for STEC were tested weekly until they were negative. Clinical data were collected through a questionnaire and by reviewing medical records.
Results. In specimens from 191 patients, *stx* was found (162 index cases). The prevalence was 1.8% in the requested group and 1.5% in the screening group ($P = .5$). Diarrhea was the most frequent symptom reported in 156 cases and of these 29 (19%) had hemorrhagic colitis (HC) and 7 children developed hemolytic uremic syndrome (HUS). No difference regarding severity of symptoms between the groups was found. *Stx2* predominated in cases with HC ($P < .0001$) and HUS ($P = .04$). Median *stx* shedding duration was 20 days (1–256 days), and no difference in duration was seen between *stx* types ($P = .106$–1.00) and presence of *eaeA* ($P = .72$).
Conclusions. Most STEC cases were found in the screening group with comparable prevalence and disease severity as in patients where analysis was requested. Furthermore, non-O157 serotypes caused severe disease when carrying *stx2*, and prolonged shedding of STEC may be a risk for transmission.

Key words. screening; serotype; shedding; Shiga toxin; STEC.

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) bacteria are causative pathogens of diarrhea, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS), and these bacteria are also the most common cause of acute renal failure in children [1–4]. Human STEC isolates are also designated enterohemorrhagic *E coli*, and serotype O157:H7 is one of the predominant serotypes responsible for outbreaks worldwide [5, 6]. Enterohemorrhagic *E coli* O157 is the main focus for diagnostics; however, non-O157 serotypes, such as O26, O103, O111, and O145, contribute significantly to cases of HC and HUS [5]. In 2011, a large outbreak of *E coli* O104:H4 in Germany led to HUS in more than 800 patients and 53 deaths [7, 8]. The Stxs are divided into 2 major types, Stx1 and Stx2, and Stx2 is responsible for the most severe symptoms [9–11]. In addition, several different subtypes of each Stx have been described [12, 13], and some Stx2 subtypes seem to be associated with more severe disease [14]. In addition, the presence of the gene for the adhesion factor intimin (*eaeA*) is linked to disease severity [9]. The issue of STEC carriage after infection is significant, and steps should be taken to limit the spread from person-to-person. However, only limited data regarding a median duration of carriage (17–18 days) [15] are available.

Journal of the Pediatric Infectious Diseases Society, Vol. 5, No. 2, pp. 147–51, 2016. DOI:10.1093/jpids/piv003

Rapid STEC detection is important in outbreak management and patient treatment, including prompt parenteral hydration, monitoring for development of severe disease, and avoidance of antibiotics and antidiarrheal agents, which can exacerbate disease [16]. The detection of STEC by culture is challenging, and traditional culture methods detect mainly O157:H7 [17, 18]. The Centers for Disease Control and Prevention (CDC) has issued recommendations to test simultaneously for O157 and non-O157 STEC in all stool specimens from patients with acute community-acquired diarrhea [19]. This diagnostic regimen was also recently recommended in a study by Lefterova et al [20]. Proper clinical diagnosis and management of non-O157 STEC infections also depend on improved physician awareness [20]. Currently, serotype-independent polymerase chain reaction (PCR) assays are used to detect *stxs* and are widely used for accurate and rapid diagnostics [20, 21].

When comparing the prevalence of STEC in the Nordic countries, Sweden shows the highest rates, with the highest number of outbreaks occurring in 2005 on the west coast of the country [22]. The higher prevalence may also depend on differences in diagnostic regimens and the number of specimens that were tested for STEC.

In this study, we evaluated a 10-year STEC PCR screening regimen in children with diarrhea in a Swedish county. The most common serotypes were correlated with clinical symptoms, *stx* type, and presence of *eaeA*. Furthermore, our goal was to add insights regarding *stx* shedding to the limited data available.

MATERIALS AND METHODS

Patients

Our study comprised all routine diarrheal stool culture specimens from patients younger than 10 years of age (n = 10 342) from 1 May 2003 through April 2013 in the County of Jönköping, Sweden. All stool specimens were collected using swabs (Copan Diagnostics Inc., Brescia, Italy). Patients were divided in 1 group in which analyses of STEC were requested by the clinician (n = 2366) and 1 screening group (n = 7976). In addition, contact tracing around index cases was performed (n = 202). The STEC-positive patients were sampled weekly until they were negative, and the duration of *stx* shedding was defined as the time from the first positive sample to the first negative sample. Clinical data were collected from all patients (n = 191) who tested positive for STEC by PCR through a questionnaire and by reviewing medical records. Results from clinical chemistry analysis on peripheral blood done at the routine chemistry laboratory at Ryhov County Hospital were available from 60 children, mainly from patients who required hospitalization. Criteria for HUS included 3 primary symptoms: hemolytic anemia with fragmentocytes, low platelet count, and acute renal failure with a creatinine outside the reference range of normal for age.

Shiga Toxin-Producing *Escherichia coli* Detection and Typing

The presence of stx in diarrheal stool specimens was determined by real-time PCR on suspensions of overnight cultures on blood agar plates [21]. In total, 157 of 191 PCR-positive specimens were sent to the Karolinska University Hospital for confirmatory testing, isolation of STEC, and serotyping according to methods described by Svenungsson et al [23].

Statistical Analyses

Statistical analyses were done with Statistica version 12. Fisher's exact test and Pearsons χ^2 test were used when comparing proportions. Mean values were compared by Mann-Whitney *U* test (nonnormal distributed) and Student *t* test as well as Bonferroni test (normal distributed). A *P* value < .05 was considered statistically significant.

RESULTS

Stx was found in specimens from 191 patients (104 boys, 87 girls), and, of these cases, 162 (85%) were index cases. Confirmatory testing at the Karolinska University Hospital, including both *stx* and *eaeA* analysis, was performed on 157 of the total 191 specimens. In 153 (97%) of these 157 specimens, STEC was confirmed by *stx* detection and culture was successful in 88 (56%) cases. In 115 of 157 (73%) specimens, *eaeA* was detected.

In total, 121 STEC cases were detected in the screening group, 41 in the requested group and 29 by contact tracing. The prevalence was 1.8% in the requested group and 1.5% in the screening group (*P* = .5), corresponding prevalence in the contact-tracing group was 14%. The numbers of children with STEC were 118 (62%), 40 (21%), and 33 (17%) in the age groups 0–3, 4–6, and 7–9 years, respectively. The annual incidence varied from 39 to 86 per 100 000 (Figure 1). Children below 10 years of age comprised 57.5% of the STEC cases in the County; however, when considering only STEC found in the requested group, children comprised approximately 20%. In comparison, culture for routine diagnostics revealed 200 cases of *Campylobacter*, 135 *Salmonella*, 76 *Yersinia*, and 18 *Shigella*, respectively.

Clinical Characteristics and Laboratory Parameters

Diarrhea was the most frequent symptom reported in 156 (82%) cases, and, of these, 29 (19%) had HC. Abdominal pain was the second most common symptom (34%), followed by vomiting (17%) and fever (17%). Seven children

developed HUS (3.6%). *Stx2* predominated in cases with HC ($P < .0001$) and HUS ($P = .04$). Hospitalization was necessary in 15 (7.8%) cases with a median length of stay of 15 days. In children with HC, elevated levels of leucocytes were detected ($P < .0001$). In HUS cases, elevated levels of leucocytes and creatinine were observed as well as low levels of thrombocytes ($P < .0001$) and erythrocyte volume fraction ($P = .0001$).

Data Regarding *stx* Shedding Time, Serotypes, and Subtypes of *stx*

Data on *stx* shedding time was available for 165 (86%) children, with a median duration of 20 days (1–256 days). For children with HC (n = 29), the median duration was 29 days (8–107 days); for children with uncomplicated diarrhea (n = 127), the median duration was 20 days (1–256 days) ($P = .07$) (Figure 2). The HUS cases had a median duration of 23 days (18–105 days). There was no difference in mean duration of *stx* shedding comparing *stx* types ($P = .11$) and presence of *eaeA* ($P = .72$).

In 88 cases, STEC isolation was successful, and the most common serotypes were O157 (n = 17), O26 (n = 14), O103 (n = 9), and O121 (n = 6). In addition, 19 other serotypes were found (Figure 3). In 3 of 7 HUS cases, isolation and serotyping was successful (two O121, one O157:H7), as well as in 15 of 29 HC cases (seven O157:H7, two O121, and two O103 and some singletons).

The distribution of *stx* types was 49%, 33%, and 18% for *stx1*, *stx2*, and *stx1* + *stx2*, respectively. We found no differences regarding *stx* types, duration of *stx* shedding, and severity of symptoms between the screening group and the requested group. *Stx1* was most frequent in serotypes O26 and O103, whereas *stx2* was more frequent in O157 and O121. Serotype O157 had a higher probability of harboring *eaeA* ($P = .004$). Patients with *stx2* were more often hospitalized ($P \leq .0001$) and had HC ($P \leq .0001$) or HUS ($P = .04$) compared with patients with *stx1* or *stx1* + *stx2*. *eaeA* showed no significant correlation with disease severity; however, HC cases showed a trend towards significance ($P = .07$). No significant difference in duration of *stx* in feces was seen between the *stx* types ($P = .106$–1.00), *eaeA* presence ($P = .72$), age groups ($P = 1.00$), or gender ($P = .35$). We found no difference between *stx* type and gender ($P = .68$) or between age groups ($P = .25$). In patients infected in Sweden, *stx2* was more common than in patients infected abroad ($P \leq .0001$).

Contact Tracing

Contact tracing was performed in approximately 112 of 162 index cases, including 202 diarrheal stool culture specimens from children below 10 years of age. In this group, the STEC-positive rate was 14% (29 of 202), which was higher than in the other 2 groups (requested and screening) (each $P < .00001$). Source of transmission was determined at 5 oc-

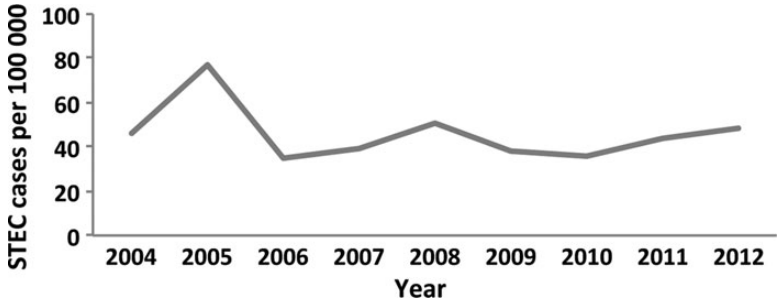

Figure 1. Annual incidence of Shiga toxin-producing *Escherichia coli* (STEC) in children below 10 years of age in the county of Jönköping, Sweden.

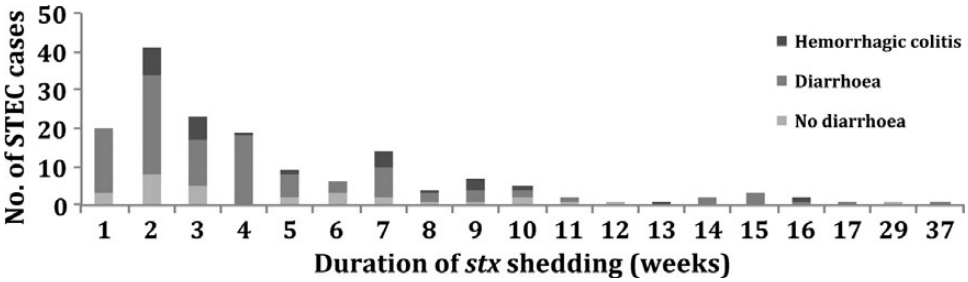

Figure 2. Duration of *stx* shedding in children with no diarrhea (n = 35), diarrhea (n = 127), and hemorrhagic colitis (n = 29). STEC, Shiga toxin-producing *Escherichia coli*.

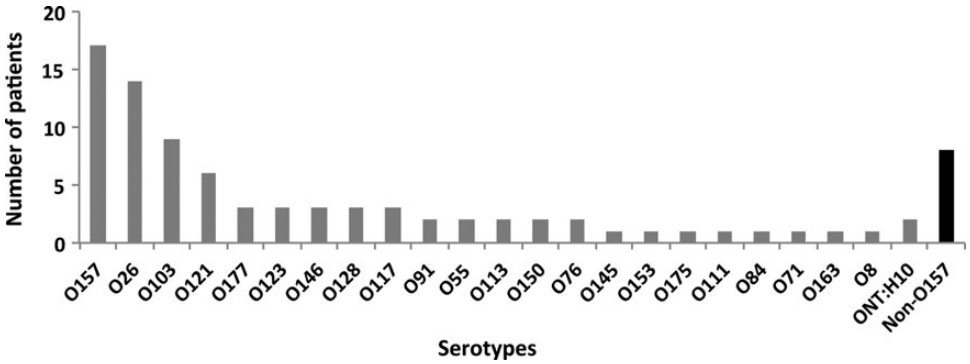

Figure 3. Serotype distribution of isolated Shiga toxin-producing *Escherichia coli* (n = 88). The black bar illustrates non-O157 strains that we were not able to serotype further.

casions; in 4 cases, it was animal contact on a farm and in one case sausage.

DISCUSSION

In this study, we show comparable STEC prevalence and disease severity in children where the analysis was not requested and in those where STEC analysis was requested. Furthermore, we found that a high diversity of serotypes, including non-O157, caused severe disease. In addition, a great variation in the duration of *stx* shedding was shown, but no relation between shedding time and *stx* type or severity of symptoms was found.

The yearly incidence was relatively constant during the study except for 2005, which coincided with an outbreak on the west coast of Sweden [22]. The southern part of Sweden, including Jönköping, generally reports the highest annual STEC figures in the country. This may be explained by higher STEC-screening activities in these counties and more farms with a higher verotoxin-producing *E coli* frequency [24]. The Swedish annual STEC incidence is higher compared with other Nordic countries [25], which may be explained by differences in diagnostic regimens, screening activities, and differences in reporting of cases between countries. In Sweden, reporting of non-O157 serotypes was included in 2004, which also includes reporting based on *stx* detection only.

One limitation of the present study is the fact that specimens were sent to another laboratory for STEC isolation, which may explain the low STEC isolation rates. However, the vast majority of *stx*-positive specimens were also detected by PCR at the Karolinska University Hospital, confirming our results. Because culture of non-O157 STEC is more challenging, we may have underestimated the importance of non-O157 STEC.

The majority of STEC cases (60%) were detected in the group of children between 0 and 4 years, which is in agreement with previous findings [26]. Children in this group often wear diapers and attend daycare, a combination that enhances the risk for transmission, as recently shown in an outbreak in Germany [27]. In accordance with previous findings, Stx2 was responsible for the most severe symptoms in our study as well [9-11]. In the present study, *stx* shedding was usually eliminated after less than 1 month; however, sometimes *stx* was excreted for several months (maximal duration 256 days). Proper follow-up of children is important to avoid further STEC spread and to prevent outbreaks. Furthermore, novel therapeutic strategies in the treatment for decolonization of long-term STEC carriers should be evaluated, and recent data indicate promising results for azithromycin [28].

In the present study, the majority of STEC cases were detected by screening of diarrhoeal stool culture specimens where analysis for *stx* was not requested. The frequent STEC detection in screening specimens underlines the lack of STEC awareness among physicians, who do not emphasize the need for STEC analysis. In recent studies, these same conclusions were reached by the CDC [19] and Lefterova et al [20]. Undetected cases of STEC infection may also be present in older individuals, and further studies are needed to determine its presence in diarrheal stool specimens of adults. Comparing culture data for routine diagnostics revealed that STEC was the second most common pathogen detected; however, because different methods are used for detection, this result should be interpreted carefully. Hence, molecular techniques can be used to detect diarrheal pathogens and affect the figures. Nevertheless, the high prevalence of STEC underlines its importance as a common diarrheal pathogen among children.

The source of infection was only revealed in 5 cases, and the methods used presently in Sweden are cumbersome, focusing only on 5 serotypes in animals. Elimination of the source is crucial in outbreak situations, and better methods are needed to determine sources of infection.

CONCLUSIONS

In conclusion, most cases of STEC were found by PCR screening, with comparable prevalence and disease severity found in patients where STEC analysis was requested. Furthermore, a high diversity of serotypes (including non-O157) caused severe disease. Serotype-independent methods for STEC detection and improved physician awareness will more accurately detect the true number of infections and enhance patient safety. Prolonged shedding of STEC may be a risk factor for transmission, and therefore local guidelines for school-aged children should be reviewed to prevent further spread.

Acknowledgments

We thank the molecular diagnostic staff at the Department of Laboratory Services in County Council Jönköping, Sweden, for continuous support.

Financial support. This work was supported by grants from Futurum, the Academy for Healthcare, County Council Jönköping, Sweden.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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