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Shiga Toxin-Producing *Escherichia coli* in Peruvian Children with Bloody Diarrhea

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are not routinely sought in clinical laboratories in developing countries. Among 131 bloody diarrhea samples in Peruvian children <5y of age, STEC was found in 9.2% and was associated with absence of fever, an observation that may increase suspicion of these pathogens. Because of the significant prevalence of STEC locally, proper diagnostics methods should be implemented in the region.

Keywords

bloody diarrhea; Shiga toxin; STEC; children; diarrheagenic *Escherichia coli*; Peru

Introduction

Bloody diarrhea is frequently treated with antibiotics without a specific etiologic diagnosis. Important infectious causes of bloody diarrhea include *Shigella*, *Salmonella*, *Campylobacter*, Shiga toxin-producing *Escherichia coli* (STEC), other diarrheagenic *E. coli* and *Entamoeba histolytica*. Management is often difficult in resource-limited settings because diagnostic methods may be unavailable or prohibitively expensive and because treatment with antimicrobial agents can be dangerous when STEC are the cause of illness. STEC, particularly serotype O157:H7, are the main cause of hemolytic uremic syndrome (HUS) (1). Use of antimicrobial agents to treat these infections might actually increase the risk of HUS (2). It is clear that STEC commonly cause illness in Argentina (3) and Chile (4) but there are fewer data regarding their frequency in other parts of South America (5). We sought to determine the prevalence and clinical characteristics of STEC infection in Peruvian children with bloody diarrhea.

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Methods

We conducted a prospective study in pediatric patients younger than 5 years of age with bloody diarrhea at the Emergency Room of Hospital Nacional Cayetano Heredia in Lima, Peru. Consecutive patients were enrolled during a period of 16 months. Epidemiologic and clinical data were collected and the patient's stool sample was taken on the first day of evaluation. Routine stool cultures were performed to determine the presence of *Shigella* and *Salmonella*. We used a multiplex real time polymerase chain reaction (RT-PCR) for detection of each category of diarrheagenic *E. coli* (6) using specific primers for the following genes: *AggR* for enteroggregative *E. coli* (EAEC), *ipaH* for enteroinvasive *E. coli* (EIEC), *eae* for enteropathogenic *E. coli* (EPEC), *lt* and *st* for enterotoxigenic *E. coli* (ETEC), *daaD* for diffusely adherent *E. coli* (DAEC) and *stx1*, *stx2* and *eae* for STEC. Additionally, an enzyme-linked immunosorbent assay (ELISA) for Shiga toxin (Stx) was performed on stools to detect Stx1 and Stx2 (Premier™ EHEC).

Results

We enrolled 131 children with bloody diarrhea between April 2007 and August 2008. The median age of the children was 15 months (range 1-69); 59% (77/131) were one year or older (Table 1). Stool cultures, RT-PCR and ELISA for STEC were done with all patient's samples. Pathogens were found in 53% (70/131) of samples; infections in which more than one organism was detected (co-infections) occurred in 15% of patients (20/131). The pathogen isolation rate was higher in children older than one year (65% vs. 37%, $p=0.002$). The most commonly isolated pathogens were *Shigella* (18%) and EPEC (11%). *Shigella* isolation was significantly more common in older children (31% vs. 0%, $p<0.001$). Fever and vomiting were significantly more frequent in children older than 1 year old, often associated with *Shigella* infections. Among 24 patients with *Shigella*, 23 (96%) had fever and/or vomiting; however, half of these patients had a co-infection with a diarrheagenic *E. coli* pathotype. The most frequent species of *Shigella* were *S. flexneri* (38%, 9/24), *S. boydii* (33%, 8/24) and *S. sonnei* (25%, 6/24).

STEC infection, measured by the presence of Shiga toxin, was detected in 12 patients (9%); the diagnosis was made by ELISA in all cases. Only 2 of these 12 patients had a positive RT-PCR in the analysis of 5 colonies; one had an *stx1*+/*eae*+ STEC strain and the other had an *stx2*+/*eae*- strain. Fever was significantly less common among STEC positive patients than patients without this pathogen (34% vs. 67%, $p<0.05$). 5 of 12 STEC-positive children (42%) had a family member with diarrhea in relation to 29/119 (24%) of STEC-negative children, although not statistically different.

Discussion

STEC isolates were identified in 9% of pediatric patients with bloody diarrhea. This rate is higher than in North American studies (7). The overall pathogen documentation was high in this study (53%) compared with other studies of bloody diarrhea. Use of several detection methods (routine culture, PCR and ELISA) resulted in recognition of STEC at a higher rate than culture on MacConkey Sorbitol media alone (8).

What distinguishes our study from others is the detection of all groups of diarrheagenic *E. coli* by routine use of PCR; this allowed definition of etiology for half of all bloody diarrhea cases. The pathogen isolation rate was significantly higher in children older than 1 year. In a recent cohort study conducted in more than 1,000 children (age range from 2 to 12 months) in Lima, Peru (9), the diarrheagenic *E. coli* as a group were the most common pathogens and their isolation was more frequent in samples from older infants, as in the current study. This

may be due to the decreased exposure to milk-related protective factors and increased exposure to contaminated food and water as dietary options becomes broader.

The diagnosis of STEC is challenging; ELISA detects the presence of the toxins with good specificity and sensitivity, although lower than cell culture (7,8). We used the Premier® EHEC ELISA which has a sensitivity of 96% and a specificity of 99% (7). In our study, only 2 of the 12 ELISA-positive samples were also positive by PCR. This discrepancy could be explained by the high frequency of antibiotic use before to the Emergency Room visit (28%). We do not recommend routine use of this method because the prevalence of STEC in watery diarrhea cases in Peruvian children is very low (9).

In our study, STEC infected children had fever significantly less often than patients without this infection ($p<0.05$), as has been reported elsewhere (7,9,10). Although the study samples were small, 5/12 patients infected with STEC had a family member with diarrhea and one of them had bloody diarrhea. This demonstrates the possibility of STEC transmission in households (10), and highlights the importance of considering family contacts for possible effective intervention for prevention of STEC infection.

Our study has several limitations; first, we may not have done optimal studies for all pathogens associated with bloody diarrhea (i.e. *Campylobacter*, *Yersinia*, *Balantidium coli*, etc). For example, our studies for *Campylobacter* may not have been optimal. However, other pathogens such as *Yersinia* and parasites associated with bloody diarrhea have historically been very rare in our setting. Second, due to the cross-sectional nature of the study we were unable to follow all of the patients to determine their outcome. This is a small study and not necessarily reflects the overall incidence of pathogens in other areas in Peru; however it is representative of the peri-urban and urban communities of Lima.

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Table 1
Clinical characteristics and pathogens isolated in bloody diarrhea patients using routine culture, RT-PCR and ELISA for STEC

Variable	< 1 year (n=54)	1 year (n= 77)	All patients (n=131)
<u>Clinical characteristics</u>			
Age, x ± sd (months)	6.4 ± 3.1	31.5 ± 17.5	21 ± 18
Gender, n% male	31 (57.4%)	28 (36.4%)	59 (47.6%)
Prior antibiotic use, n%	15 (27.8%)	22 (28.6%)	37 (28.2%)
Disease duration [¥] , x ± sd (days)	3.1 ± 2.9	3.1 ± 2.9	3.1 ± 2.9
Fever, n%	27 (50%)	55 (71.4%) *	82 (62.6%)
Vomiting, n%	19 (35.2%)	35 (45.5%) *	54 (41.2%)
Blood in stools during examination [£] , n%	51 (94.4%)	69 (89.6%)	120 (91.6%)
Relative with diarrhea, n%	12 (22.2%)	22 (28.6%)	34 (26.0%)
Relative with bloody diarrhea, n%	6 (11.1%)	7 (9.1%)	13 (9.9%)
<u>Pathogens</u>			
<i>E. coli</i> [†]	21 (38.9%)	45 (58.4%) *	66 (50.4%)
STEC ^π	5 (9.3%)	7 (9.1%)	12 (9.2%)
EPEC	6 (11.1%)	8 (10.4%)	14 (10.7%)
ETEC	0 (0%)	10 (13.0%)	10 (7.6%)
DAEC	4 (7.4%)	6 (7.8%)	10 (7.6%)
EAEC	5 (9.3%)	2 (2.6%)	7 (5.3%)
EIEC	1 (1.9%)	12 (15.6%)	13 (9.9%)
<i>Shigella</i>	0 (0%)	24 (31.2%) **	24 (18.3%)
<i>Salmonella</i>	0 (0%)	2 (2.6%)	2 (1.5%)
Co-infections [‡]	1 (1.9%)	19 (24.7%)	20 (15.3%)
Negative to all pathogens	34 (63%)	27 (35.1%) **	61 (46.6%)

* p < 0.05;

** p < 0.01;

[¥] Prior to the Emergency Room visit;

[†] Diarrheagenic *E. coli*.

[£] Macroscopic blood in stools during examination;

^π STEC was the only pathogen in which a combination of techniques (PCR and ELISA) were used to define prevalence;

[‡] Co-infections: *Shigella* with diarrheagenic *E. coli* (14 patients); co-infection among the diarrheagenic *E. coli* groups (6 patients).