INTESTINAL BLEEDING AND OCCLUSION ASSOCIATED WITH SHIGA TOXIN-PRODUCING
ESCHERICHIA COLI O127: H21

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Abstract
We report a case of a nine-year old boy with vomiting, abdominal pain and fever, who underwent surgery with a diagnosis of appendicitis in Mendoza and from whom a Shiga toxin-producing Escherichia coli (STEC) O127:H21 strain was recovered. Forty-eight hours after surgery he presented bilious vomiting and two episodes of intestinal bleeding. Laboratory findings included: hematocrit, 35%; blood urea nitrogen, 0.22 g/L. The urinary output was normal. The following day physical examination showed an alert mildly hydrated child, without fever but with distended and painful abdomen. The patient was again submitted to surgery with a diagnosis of intestinal occlusion. Bleeding and multiple adhesions in jejunum and ileum were found. The patient still had tense and painful abdomen and presented two bowel movements with blood; hematocrit fell to 29% and blood urea nitrogen rose to 0.32 g/L. STEC O127:H21 eae(-)/Stx2/Stx2vh-b(+)/E-Hly(+) was isolated from a stool sample. He was discharged after 10 days of hospitalization and no long-term complications such as HUS or TTP were observed. This is the first report, to our knowledge, on the isolation of E. coli O127:H21, carrying the virulence factors that characterize STEC strains, associated to an enterohemorrhagic colitis case. This serotype was previously characterized as a non-classic enteropathogenic E. coli (EPEC). STEC infections can mimic infectious or noninfectious pathologies. Therefore an important aspect of clinical management is making the diagnosis using different criteria thereby avoiding misdiagnoses which have occasionally led to invasive diagnostic and therapeutic procedures or the inappropriate use of antibiotics.

Resumen
Hemorragia y oclusión intestinal asociadas a Escherichia coli O127:H21 productor de toxina Shiga. Se presenta el caso de un niño de 9 años, eutrófico, perteneciente a una familia de bajo nivel socioeconómico en Mendoza, que ingresó al hospital con vómitos, fiebre y dolor abdominal y al cual se le practicó apendectomía, aislándose Escherichia coli O127:H21, productor de toxina Shiga (STEC) en el coprocultivo. A las 48 horas post-cirugía, el paciente presentó vómitos biliosos y dos episodios de hemorragia intestinal. El hematocrito fue de 35% y la ureemia de 0.22 g/L. La diuresis fue normal. Al día siguiente estaba afebril, alerta, hidratado, pero con el abdomen distendido y doloroso. Fue enviado nuevamente a cirugía con diagnóstico de oclusión intestinal, observándose durante la misma, sangrado intestinal y múltiples adherencias en yeyuno e íleo, que fueron manualmente removidas. El paciente continuó con distensión abdominal y presentó dos deposiciones sanguinolentas, el hematocrito disminuyó a 29% y la urea en sangre se elevó a 0.32 g/L. STEC O127:H21 eae(-)/Stx2/Stx2vh-b(+)/E-Hly(+) fue aislado de la materia fecal. El niño fue dado de alta después de 10 días de hospitalización sin presentar complicaciones como SUH o PTT. Esta es la primera comunicación sobre el aislamiento de E. coli O127:H21 de un caso de enterocolitis hemorrágica, con los factores de virulencia que caracterizan a STEC. Este serotipo era reconocido como perteneciente a la categoría de E. coli enteropatógeno no clásico. Teniendo en cuenta que las infecciones por STEC pueden mimetizar otras patologías infecciosas o no infecciosas, es importante utilizar distintos criterios diagnósticos, para evitar prácticas médicas o tratamientos innecesarios.

Key words: Escherichia coli O127:H21 infection, intestinal bleeding, intestinal occlusion

Shiga toxin-producing Escherichia coli (STEC), previously referred to as Shiga-like toxin or verocytotoxin–producing E. coli1, is the causative agent of hemorrhagic colitis (HC), a bloody diarrhea that usually occurs without fecal leucocytosis or fever. E. coli O157:H7 is the predominant serotype isolated in outbreaks of HC in many countries, including the USA, UK, Canada and Japan. However, more than 25 other serotypes (O5:NM; O26:H11; O111:H8; O113:H21; O128:NM; O145:NM; among others) that share a similar pathogenic potential, have been isolated from HC cases2. The group is called enterohemorrhagic E. coli (EHEC).

The illness typically begins with severe abdominal cramps and non-bloody diarrhea, which may become grossly bloody by the second or third day of illness. About
half the patients have nausea and vomiting. Other infectious causes of diarrhea (Shigella, Salmonella, Campylobacter, Yersinia enterocolitica, Clostridium difficile) must be considered in making the diagnosis. However, unlike most bacterial enteric diseases, a low-grade fever or the absence of fever usually characterizes O157:H7 infection. This feature may lead clinicians to suspect noninfectious causes, such as inflammatory bowel disease, ischemic colitis, or in children, intussusception. Because the abdominal pain and tenderness may be severe, an acute condition requiring surgery may be the initial diagnosis, leading to exploratory laparotomy.

Stools from patients with E. coli O157:H7 infection are sometimes described as all blood and no stool simulating gastrointestinal hemorrhage. Edema and submucosal hemorrhage in the ascending and transverse colon may be demonstrated by a thumbprinting pattern on barium-enema examination. At endoscopy, the colonic mucosa appears edematous and hyperemic, sometimes with superficial ulcerations. Pseudomembranes may be present making it difficult to distinguish between C. difficile pseudomembranous colitis and infection with STEC.

Numerous epidemiological studies have demonstrated an association between HC and STEC infection. Life threatening systemic complications can also result from infection including the hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). A triad of acute renal failure, microangiopathic hemolytic anemia and thrombocytopenia characterizes HUS. Patients with HUS are also at increased risk of neurological symptoms including lethargy, severe headache, convulsions and encephalopathy. HUS following STEC infection is recognized as the most common cause of pediatric acute renal failure in many countries, including Argentina. TTP, which is identical in its clinicopathological features to HUS, occurs more often in infected elderly patients. In addition, more prominent central nervous system abnormalities are reported to occur with TTP.

STEC strains elaborate potent bacteriophage-encoded cytotoxins called Shiga toxins (Stxs). There are two main types of Stx: Stx1 and Stx2, and within the Stx2 family there are various sub-types produced by human STEC isolates. The toxins may be present alone or as a combination of two toxins (typically Stx1; Stx2 or Stx2/ Stx2v). Apart from Stx, EHEC strains generally express other virulence-associated factors, which may be linked to their ability to cause severe disease. These factors include a novel hemolysin called enterohemolysin (EHEC-Hly) encoded by a 60MDa plasmid (pO157), and intimin, an outer membrane protein encoded by the chromosomal eae gene that is involved in intimate attachment to its receptor (Tir) on the host cell and effacement of microvilli of colonic mucosa (AE lesion). Both genes (eae and tir) are located in the locus of enterocyte effacement (LEE) pathogenicity island.

At present, there is no vaccine to protect against STEC infection nor are there effective interventions to treat STEC-induced diseases. In fact, antibiotic therapy may be a risk factor for developing complications of STEC infection because antibiotics increase toxin release from the periplasm of the bacterium. Current therapy for STEC-associated illnesses consists of supportive care.

We report a case of intestinal bleeding and occlusion associated with STEC O127:H21, a serotype previously considered a non-classic enteropathogenic E. coli (EPEC), in a well nourished child nine-years old, belonging to a low socioeconomic family. He was admitted to the Hospital Regional Antonio J. Scaravelli, Mendoza, Argentina, on 19 August 1995 with vomiting, abdominal pain and fever. Appendectomy was performed after clinical diagnosis of acute appendicitis. No pathologic studies were carried out. Twenty-four hours after surgery, the patient presented abdominal distention with generalized pain without fever and vomiting. Plain abdominal radiograph demonstrated multiple air-fluid levels throughout the abdomen with thickening of the bowel wall in a child with intestinal bleeding and occlusion associated with Shiga toxin-producing Escherichia coli O127:H21.
levels throughout the abdomen with thickening of the bowel wall. Nasogastric and rectal decompression was done. Forty-eight hours later, he presented bilious vomiting and had suffered two episodes of intestinal bleeding, of around 300 ml each at 72 hours.

Laboratory findings included: White blood cells (WBC) count, 7,100/ml; hematocrit, 35%; prothrombin, 85%; blood urea nitrogen, 0.22 g/L. The urinary output was normal. Therapy with ampicillin 100 mg/kg/day and gentamicin 5 mg/kg/day was started. Rectal digital examination showed an empty rectum for feces, it was followed for a discharge of fresh blood. A new plain abdominal radiograph was requested and the patient was immediately referred to the Hospital Pediátrico Dr. Humberto J. Notti on 22 August. Physical examination showed an alert mild hydrated child, without fever but with distended and painful abdomen. He was treated with penicillin 100,000 U/kg/day, gentamicin 5 mg/kg/day and metronidazole 25 mg/kg/day, WBC count was 7,200/ml; hematocrit, 33%; platelet count, 280,000/ml and blood urea nitrogen, 0.22 g/L.

Plain abdominal radiograph showed a progressive distention (Fig. 1) but abdominal sonography, barium- enema examination and/or abdominal computed tomography scan were not considered in making the diagnosis. The patient was submitted to surgery with presumptive diagnosis of intestinal occlusion. During exploratory laparotomy, intestinal bleeding and multiple adhesions in jejunum and ileum were found. They were manually liberated and after surgical procedure intestinal motility returned to normal. The patient still had tense and painful abdomen and presented two bowel movements with blood in feces on 23 August. Hematocrit fell to 29% and blood urea nitrogen raised to 0.32 g/L. After forty-eight hours the patient was in good conditions with only minimal blood in feces and 24 hours later the antimicrobial therapy was suspended. The patient recovered from the infection and did not go to develop either HUS or TTP. He was discharged to his home on 29 August.

A fecal sample was collected on 23 August. In addition to be cultured for E. coli, the stool was also routinely cultured for Salmonella, Shigella, Yersinia, Vibrio, Campylobacter, and Clostridium species with negative results. The stool was not evaluated for C. difficile toxins A and B. Sorbitol-fermenting colonies were observed on sorbitol-MacConkey agar. Biochemical reactions were positive for β-glucuronidase, indole production, methyl red test, glucose acid production, D-mannitol, raffinose and trehalose fermentation, lysine and ornithine decarboxylase. Six colonies identified as E. coli were positive for Stx2 production in cytotoxic assays on Vero cells using Stx1 and Stx2 specific monoclonal antibodies (MAb 13C4 and BC5BB12, respectively), provided by NA Stockbaine, Center for Infectious Diseases, Atlanta, GA.

The strains were positive for stx2 gene and EHEC virulence plasmid analyzed by colony blot hybridization assays with specific digoxigenin labeled gene probes (provided by Drs J Kaper and J Nataro, Center for Vaccine Development, University of Maryland, School of Medicine, Baltimore, MA) under stringent conditions. The strains were negative for the eae factor and the bundle forming plus (bfp) and did not adhere to HeLa cells. The PCR test for amplification of stx gene sequences was performed with the oligonucleotide primers described by Pollard et al. Amplified products of the stx2 gene (346-bp fragment) were obtained. The strains were characterized as Stx2/ Stx2vh-b by the PCR-RFLP (Restriction Fragment Length Polymorphism) genotyping method of Tyler et al.

The strains were positive for E-Hly on the sheep blood agar assay. They were serotyped by agglutination with specific O and H antisera (Denka Seiken CO., Ltd., Japan) and identified as E. coli O127:H21. They were susceptible to ampicillin, cefixime, cefotaxime, cefuroxime, cefepime, cephalotin, colistin, chloramphenicol, gentamicin, nalidixic acid, norfloxacin, streptomycin and tetracyclin as determined by Kirby Bauer method.

Human pathogenicity of E. coli strains belonging to the STEC group varies according to serotypes, virulence attributes and others unknown factors. Serotype O127:H21 carrying the virulence factors than characterize STEC strains, as far as we know, has not been previously reported as a cause of HC.

E. coli O127:H21 eae(-)/Stx2/Stx2vh-b(+)/E-Hly(+)) was isolated from a child with diagnosis of appendicitis, who suffered intestinal bleeding and occlusion and underwent two surgeries. He did well following the discharge and no long-term complications such as HUS or TTP were observed.

E. coli O127:H21 was previously characterized as a non-clasric EPEC with the ability to cause gastroenteritis in very young children and rarely in adults. On the other hand, EPEC are found in human populations throughout the world and constitute one of the main causes of infantile diarrhea in developing countries, including Argentina.

A new STEC serotype could conceivably have arisen when an E. coli O127:H21 strain became lysogenized with one or more Stx-encoding bacteriophages or acquired virulence factors, such as Shiga-toxin, plasmid-encoded adhesins and enterohemolysin, via horizontal transfer and recombination from an EHEC strain. The direct involvement of such mobile elements in transmission of stx genes has yet to be demonstrated. However, one study has identified an IS element adjacent to an stx1 operon in an O111:H STEC strain, there was no duplication of target sequence at the insertion site, which raised the possibility that the segment of DNA containing the toxin gene was part of a transposon. Involvement of bacteriophages and transposons may help
to explain why many STEC strains readily lose their stx genes after subcultivation in vitro.

STEC infections can mimic infectious or noninfectious pathologies. Therefore an important aspect of clinical management is making the diagnosis using different criteria such as colonic biopsy, abdominal sonography, barium-enema examination and/or abdominal computed tomography scan, thereby avoiding misdiagnoses which have occasionally led to invasive diagnostic and therapeutic procedures or the inappropriate use of antibiotics.

Acknowledgements: The authors are grateful to Dr. Luis E. Voyer, for helpful discussion and review of the manuscript. The technical assistance of Ana Garbini and Natalia Martínez is acknowledged. This work was partially supported by a grant from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) (PIA N° 7237/97).

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