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 uremia 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. coli* [1], 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 cases [2]. 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[3].

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[4].

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[5]. 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[2].

STEC strains elaborate potent bacteriophage-encoded cytotoxins called Shiga toxins (Stx_s)[1]. 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)[6] encoded by a 60MDa plasmid (pO157), and intimin, an outer membrane protein encoded by the chromosomal *eae* gene[7] 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[8] 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

Fig. 1.– Plain abdominal radiograph demonstrating 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 derived 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[9] using Stx1 and Stx2 specific monoclonal antibodies (MAb 13C4 and BC5BB12, respectively), provided by NA Strockbine, Center for Infectious Diseases, Atlanta, GA.

The strains were positive for *stx*2 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[10]. The strains were negative for the *eae* factor and the bundle forming pilus (*bfp*) and did not adhere to HeLa cells[11]. The PCR test for amplification of *stx* gene sequences was performed with the oligonucleotide primers described by Pollard et al[12]. Amplified products of the *stx*2 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[13].

The strains were positive for E-Hly on the sheep blood agar assay[6]. 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, 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-clasic 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[14].

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 *stx*1 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 bacteriohages and transposons may help

to explain why many STEC strains readily lose their *stx* genes after subcultivation *in vitro*[15].

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.

References

1. Calderwood SB, Acheson DWK, Keusch GT, Barrett TJ, Griffin PM, Strockbine NA et al. Proposed New Nomenclature for SLT (VT) family. *ASM News* 1996; 62: 118-9.
2. Griffin P, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *Escherichia coli,* and the associated hemolytic uremic syndrome. *Epidemiol Rev* 1991; 13: 60-98.
3. Tarr P. *Escherichia coli* O157:H7: Clinical, Diagnostic and Epidemiological aspects of Human infection. *Clin Infect Dis* 1995; 20: 1-10.
4. Boyce TG, Swerdlow DL, Griffin PM. *Escherichia coli* O157:H7 and the Hemolytic-Uremic Syndrome. *N Engl J Med* 1995; 333: 363-8.
5. Rivas M, Balbi L, Miliwebsky ES, García B, Tous M, Leardini N, et al. Síndrome urémico hemolítico en niños de Mendoza, Argentina. Asociación con la infección por *Escherichia coli* productor de toxina Shiga. *Medicina (Buenos Aires)* 1998; 58: 1-7.
6. Beutin L, Zimmermann S, Gleier K. Rapid detection and isolation of Shiga-like toxin (verocytotoxin)-producing *Escherichia coli* by direct testing of individual enterohemolytic colonies from washed sheep blood agar plates in the VTEC-RPLA assay. *J Clin Microbiol* 1996; 34: 2812-4.
7. Yu J, Kaper JB. Cloning and characterization of the *eae* gene of enterohemorrhagic *Escherichia coli* O157:H7. *Mol Microbiol* 1992; 6: 411-7.
8. Proulx F, Turgeon JP, Delage G, Lafleur L, Chicaine L. Randomized controlled trial of antibiotic therapy for *Escherichia coli* O157:H7 enteritis. *J Pediatr* 1992; 121: 299-303.
9. Karmali MA, Petric M, Lim C, Cheung R, Arbus GS. Sensitive method for detecting low numbers of Verotoxin-producing *Escherichia coli* in mixed cultures by use of colony sweeps and polymyxin extraction of Verotoxin. *J Clin Microbiol* 1985; 22: 614-9.
10. Thomas A, Smith HR, Wilshaw GA, Rowe B. Non-radioactively labelled polynucleotide and oligonucleotide DNA probes, for selectively detecting *Escherichia coli* strains producing Vero cytotoxins VT1, VT2 and VT2 variant. *Mol Cell Prob* 1991; 5: 129-35.
11. Scaletsky ICA, Silva MLM, Trabulsi LR. Distinctive patterns of adherence of enterohemorrhagic *Escherichia coli* to HeLa cells. *Infect Immun* 1984; 45: 534-6.
12. Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR. Rapid and specific detection of Verotoxin genes in *Escherichia coli* by the Polymerase Chain Reaction. *J Clin Microbiol* 1990; 28: 540-5.
13. Tyler SD, Johnson WM, Lior H, Wang G, Rozee KR. Identification of Verotoxin Type 2 variant B subunit genes in *Escherichia coli* by the Polymerase Chain Reaction and Restriction Fragment Length Polymorphism analysis. *J Clin Microbiol* 1991; 29: 1339-43.
14. Vergara M, Quiroga M, Grenon S, et al. Prospective study of enteropathogens in two communities of Misiones, Argentina. Rev Inst Med Trop São Paulo 1996; 38: 337-47.
15. Paton AW, Paton JC. Characterization of IS 1203, an insertion sequence in *Escherichia coli* O111:H⁻. *Gene* 1994; 150: 67-70.

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Creo que la fortuna es árbitra de la mitad de nuestras acciones, pero nos deja gobernar la otra mitad a nosotros.

Nicolás Maquiavelo (1469-1527)

El príncipe