Neonatal Hemolytic Uremic Syndrome After Mother-to-Child Transmission of a Low-Pathogenic *stx*2b Harboring Shiga Toxin–Producing *Escherichia coli*

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This case describes evidence for a Shiga toxin–producing *Escherichia coli* (STEC) O146:H28 infection leading to hemolytic uremic syndrome in a neonate. STEC O146:H28 was linked hitherto with asymptomatic carriage in humans. Based on strain characteristics and genotyping data, the mother is a healthy carrier who transmitted the STEC during delivery. STEC strains belonging to the low-pathogenic STEC group must also be considered in the workup of neonatal hemolytic uremic syndrome.

Keywords. hemolytic uremic syndrome; newborn; neonatal; Shiga-toxin producing *Escherichia coli*; STEC.

The hemolytic uremic syndrome (HUS) is a rare and severe thrombotic microangiopathy (TMA) characterized by the triad of hemolytic anemia, thrombocytopenia, and acute renal failure. In >90% of cases, the disease is triggered by an infection with Shiga toxin–producing *Escherichia coli* (STEC) harboring specific Shiga toxin (Stx) 2 subtypes and the adhesion factor intimin and often presents with a prodromal bloody diarrhea. In particular, children between 2 and 6 years of age are sensitive to the development of the disease. This HUS is called typical HUS or STEC-HUS [1]. Non-STEC-HUS or atypical HUS (aHUS) is seen in 5%–10% of all HUS cases and can appear at any age and may be sporadic or familial. The majority of cases of a HUS are caused by a disorder in complement alternative pathway regulation and aHUS is often used to make referral to this particular subgroup [2].

Diagnosis of aHUS relies on (1) no associated disease, (2) no criteria for STEC-HUS, and (3) no criteria for thrombotic thrombocytopenic purpura (TTP). Even aHUS and TTP overlap clinically and morphologically; the discovery of the specific involvement of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) in the pathogenesis of TTP allows discrimination between the 2 TMAs: in patients with TTP, ADAMTS13 activity is greatly reduced (5%–10% of normal) [3].

Neonatal cases of HUS are predominantly related to aHUS (in particular metabolic diseases like cobalamin C disorders); yet, neonatal cases of HUS due to Shiga toxin–producing *E. coli* have been anecdotally reported [4, 5].

We describe the case of a newborn boy presenting with HUS associated with a low-pathogenic STEC. Genetic analyses of the STEC strains were able to clarify the route of infection.

CASE REPORT

A male newborn (weight: 3680 g, 50th centile; length: 52 cm, 50th–90th centile; head circumference: 34 cm, 10th centile) was born vaginally by a healthy mother at 41 2/7 weeks of gestation after a normal pregnancy. Neonatal adaptation was normal (Apgar score: 7/9/10) and first micturition and meconium were unremarkable in the first 24 hours of life. Two days after birth, the newborn displayed relapsing biliary vomiting in absence of diarrhea or blood-stained stool losses. Ileus, volvulus, and malrotation were ruled out by abdominal radiography and ultrasound. Laboratory investigations did not show signs of infection or an underlying metabolic disease. Vomiting stopped spontaneously after 48 hours; oral feeding was restarted and well tolerated.

At day 6 of life, a sudden increase of bilirubin (total bilirubin: 374 µmol/L, conjugated bilirubin: 42 µmol/L, normal values <4 µmol/L) was observed and phototherapy started. Further laboratory investigations revealed the presence of TMA with hemolytic anemia (hemoglobin, 111 g/L [normal range, 137–205 g/L]; lactate dehydrogenase, 2223 IU/L [normal value, <976 IU/L]; fragmentocytes), thrombocytopenia (39 G/L

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[normal range, 100–400 G/L]), and acute renal failure (creatinine, 101 μmol/L [normal value, <78 μmol/L]; urea, 16 mmol/L [normal range, 1.4–6.8 mmol/L]). Urinalysis showed pathologically increased proteinuria and hematuria, and blood pressure was within the normal range. Twenty-four hours later, the newborn developed several episodes of epileptic seizures, which were successfully treated with phenobarbital and topiramate. Cerebral ultrasound was normal and electroencephalography registered multifocal epileptogenic activity.

Because the newborn did not show any additional deterioration of renal function and recovered quickly with normalization of the hematological and renal parameters together with stable neurological condition without seizures within 48 hours, renal replacement therapy, plasma exchange, or the monoclonal antibodies against terminal complement protein C5 eculizumab were not prescribed.

Testing for causes of atypical HUS and TTP (metabolic, complement factors, and ADAMTS13 activity) remained negative. Yet, fecal analysis disclosed the presence of STEC, thus permitting the diagnosis of typical HUS. Family history was negative for renal diseases, parents are not consanguineous, and neither parent had gastrointestinal symptoms during the previous 3 weeks.

At the 11th day of life, the newborn was discharged from hospital in good general condition and with normal laboratory findings. Antiepileptic therapy with phenobarbital and topiramate was tapered during the next 3 months. Within the last 9 months of follow-up, hematological and renal parameters remained within normal range, and the somatic and neurological development was unremarkable.

MICROBIOLOGICAL ANALYSIS

Stool samples from the boy and the mother were negative for *Salmonella*, *Campylobacter*, and *Shigella* by standard bacteriology [6] but positive by using an enzyme immunoassay (ProSpecT Shiga Toxin *E. coli* test; Remel, Lenexa, Kansas) as well as multiplex polymerase chain reaction (PCR) analysis (*E. coli* DEC Primer Mix, Statens Serum Institut, Copenhagen, Denmark) for STEC.

For the isolation of STEC strains, the stool samples were streaked onto MacConkey agar, and, after incubation at 37°C for 24 hours, colonies were washed off with 2 mL of 0.85% saline solution and tested by colony dot-blot hybridization with Shiga toxin DNA probes as described by Kaufmann et al [7]. One isolate per sample, corresponding to colored spots, was picked from the hybridization plate, grown at 37°C overnight on sheep blood agar, and confirmed as STEC.

Serotyping of the strains was performed with O (O1 to O186) and H (H1 to H56) specific rabbit antisera produced at the Federal Institute for Risk Assessment in Germany.

Table 1. Presence of Selected Non-*stx* Putative Virulence Genes Including Toxins, Adhesins, and Plasmid-Located Genes for the 2 Shiga Toxin–Producing *Escherichia coli* Strains Isolated From a Newborn Boy (N12-0034) and His Mother (N12-0035)

Gene	Description	N12-0034 (Neonate)	N12-0035 (Mother)
EHEC-*hlyA*	EHEC hemolysin	–	–
astA	Heat-stable enterotoxin 1	+	+
cdtB	Cytolethal distending toxin B	–	–
eae	Intimin	–	–
iha	Iron-regulated gene A homologue adhesion	+	+
efa-1	EHEC factor for adherence	–	–
saa	Auto agglutinating adhesin	–	–
lpfA	Long polar fimbriae	+	+
bfpA	Major subunit of bundle-forming pili	–	–
katP	Catalase-peroxidase	–	–
espP	Serine protease	–	–
etpD	Type II secretion system	–	–

+, the gene is present; –, the gene is absent.

Abbreviation: EHEC, enterohemorrhagic *Escherichia coli*.

Shiga toxin genes were subtyped by PCR according to a standard procedure proposed by the World Health Organization Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* (http://www.ssi.dk). Genes encoding non–Shiga toxin putative virulence factors of STEC, including toxins, adhesins, and plasmid-located genes (Table 1), were detected using a DNA microarray (Identibac *E. coli* Genotyping, Alere, Germany).

For genotyping, pulsed-field gel electrophoresis (PFGE) was performed by following the Centers for Disease Control and Prevention PulseNet protocol using the restriction enzyme *XbaI* and the CHEF-DR III system (Bio-Rad Laboratories). The pulse times were ramped from 5 seconds to 50 seconds for 19 hours and an angle of 120°. *Salmonella* Braenderup strain H9812 (ATCC BAA 664) was used as reference.

RESULTS OF STEC CHARACTERIZATION

The 2 STEC strains isolated from the neonate (strain N12-0034) and the mother (strain N12-0035) showed a sorbitol-fermenting STEC serotype O146:H28 that harbored the virulence gene *stx*2, but were negative for *eae* and *hlyA*. Selected results for further virulence-associated genes are

summarized in Table 1. Both strains produced Shiga toxin as detected by the enzyme-linked immunosorbent assay. Typing the *stx*2 genes revealed for both strains the variant *stx*2b. In addition, the strains of the mother and her neonate were indistinguishable in the PFGE analysis.

DISCUSSION

We describe clinical evidence for an STEC O146:H28 infection leading to HUS in a neonate at the sixth day of life. STEC O146:H28 was linked so far with asymptomatic carriage in humans [8, 9].

Pathogenicity of STEC is associated with various virulence factors, the most important of which are the Shiga toxins. These toxins can be subdivided into 2 main groups, Stx1 and Stx2 [10]. STEC strains pathogenic for humans tend to feature Stx2 and other virulence traits as the adhesion factor intimin [11]. Of the 7 Stx2 variants described so far, the representatives of the Stx2acd group (*stx2*a, *stx2*c, *stx2*d), which are genetically closely related, are reported to be strongly associated with HUS in patients [11, 12]. The isolated strains, however, harbored the variant *stx*2b and were *eae* negative, which has been linked with mild clinical signs or asymptomatic carriage [13, 14].

The mother had no gastrointestinal symptoms and the newborn presented with vomiting without diarrhea 4 days before developing hemolytic anemia and renal failure. Based on the strain characteristics and the genotyping data, we postulate that the mother is a healthy carrier, who transmitted the STEC by the fecal-oral route to the newborn during delivery. In a newborn's sterile bowel, this STEC may be able to proliferate and lead to HUS.

In newborns presenting with HUS, it is important to rule out all causes that can lead to atypical HUS. Even though infectious HUS is a very rare condition in this age group, the search for this entity is mandatory. STEC strains belonging to the low-pathogenic STEC group should also be considered.

Notes

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