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# Recurrent Hemolytic and Uremic Syndrome Induced by Escherichia Coli

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**Abstract:** A widespread belief is that typical hemolytic and uremic syndrome (HUS) does not recur. We report the case of a patient infected twice with raw milk taken from his own cow and containing a Shiga toxin–producing *Escherichia coli* O174:H21 that induced recurrent HUS causing severe renal and cerebral disorders. A genomic comparison of the human and bovine Shiga toxin–producing *Escherichia coli* O174:H21 isolates revealed that they were identical.

Typical HUS may recur. Since milk from this animal was occasionally distributed locally, thereby posing a serious threat for the whole village, this particular cow was destroyed.

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**Abbreviations**: ADAMTS-13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13, HUS = hemolytic and uremic syndrome, PFGE = pulsed-field gel electrophoresis, STEC = Shiga toxin–producing *Escherichia coli*, TMA = thrombotic microangiopathy.

## CASE REPORT

A 76-year-old man was admitted to our renal emergency unit for the recurrence of a thrombotic microangiopathy (TMA). The first episode had been diagnosed 8 months earlier, shortly after the patient was back from Tinghir, his hometown in rural Morocco. At that time, TMA was ''atypical'' in that the patient had no diarrhea, and was complicated by acute renal failure and generalized seizures. The activity of a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13) was moderately decreased (17%), which ruled out thrombotic thrombocytopenic purpura. Serum concentration of complement proteins was in the normal range (C3

fraction: 1150 mg/L, C4: 290 mg/L), as was that of factor B (157 mg/L), H (107% of controls), and I (141%). Further, no mutation was found in *CFH, CFHR, CFI, CD46* genes, respectively, encoding complement factor H, complement factor H–related proteins, complement factor I, and MCP proteins of the alternative pathway, and currently known to cause atypical hemolytic and uremic syndrome (HUS). Similarly, no complex rearrangement was found between *CFH* and *CFHR* genes. Initial treatment consisted of a total of 14 plasma exchanges, after which he fully recovered (at that time eculizumab was not approved for the treatment of atypical HUS by the European Food Safety Authority, and hence was not considered here). The second episode of TMA occurred, again after a trip to Tinghir, yet this time accompanied by bloody diarrhea. Temperature was 36°C and arterial pressure 112/73 mmHg. Clinical examination was unremarkable. Platelet count was 58,000/mm$^3$, Hb 9.7 g/dL with 1.8% of schistocytes, lactate dehydrogenase 857 UI/L, haptoglobin < 0.08 g/L, and serum creatinine 237 μmol/L. Of note, during this second flare of TMA the enzymatic activity of ADAMTS-13 was also preserved and was 55% by the fluorescence-quenching substrate for ADAMTS-13 assay and 30% by using a method that tests the full-length protein; no ADAMTS-13 binding antibodies were detected. A rectosigmoidoscopy revealed a colitis, and a biopsy sample yielded a Shiga toxin–producing *Escherichia coli* (STEC). Although recurrence of HUS is rare, the coincidence of his return to Morocco and the occurrence of HUS on 2 different occasions was striking. An in-depth interview of the patient revealed that he had kept a cow in his yard at Tinghir for several years. He milked his own cow, and would then drink the raw, unpasteurized milk. To test whether the cow was the reservoir of STEC, we asked his family to provide us with raw milk and feces from the cow. Cow feces and milk samples were collected directly from the cow, kept at +4°C, and sent to the French National Reference Laboratory for *E coli* (VetAgro Sup, Marcy l'Etoile). All samples from the cow (4 fecal and 4 raw milk samples of 25 mL each) were analyzed according to the protocol described in ISO TS 13136:2012 (Anonymous: 2012). Briefly, this protocol uses real-time polymerase chain reaction to detect food-borne pathogens and a horizontal method for the detection of STEC and the determination of O157, O11, O26, O103, and O145 serogroups. One STEC was isolated from a milk sample.

Polymerase chain reaction screening for STEC virulence factor genes in both the colon biopsy sample and the cow's milk isolate was positive for *stx1* and *stx*2 (respectively, *stx1a* and *stx2a* subtypes), EHEC-*hlyA* (coding for hemolysin A), and *saa* (coding for STEC autoagglutinating adhesin) genes, whereas *eae* (coding for intimin) and *agg*R (which regulates the expression of aggregative adherence fimbriae in enteroaggregative *E coli*) genes were negative.[1,2] Antibiotic susceptibility determined by the disk diffusion method with
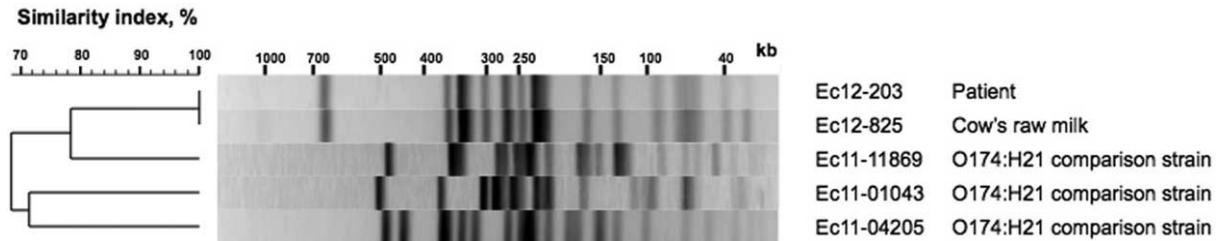
**FIGURE 1.** Dendrogram generated by BioNumerics version 4.1 (Applied Maths, Sint-Martens-Latem, Belgium) showing the results of cluster analysis on the basis of *XbaI* of 5 *E coli* O174:H21 isolates. Similarity analysis was performed by using the Dice coefficient, and clustering was done by using the unweighted pair-group method with arithmetic averages. The origin of the isolates is shown. *E coli* = *Escherichia coli*, PFGE = pulsed-field gel electrophoresis.

32 antimicrobial drugs (Bio-Rad, Berkeley, CA) revealed that both isolates had the same resistance pattern (resistance to ampicillin, streptomycin, sulfonamides, trimethoprim, and cotrimoxazole, but susceptibility to third-generation cephalosporins, nalidixic acid, ciprofloxacin, kanamycin, gentamicin, chloramphenicol, tetracycline, and azithromycin). Conventional O serotyping with a limited set of antisera found an agglutination with polyvalent A and O18 antisera (Sifin, Berlin, Germany). However, molecular serotyping based on the analysis of the O-antigen gene cluster and flagellin gene sequencing found that both isolates belonged to the O174:H21 serotype.[3] Multilocus sequence typing revealed that both isolates were sequence type 677.[4] Finally, genomic comparison of the human and bovine STEC O174:H21 isolates, as well as 3 other STEC O174:H21 isolated in 2011 but epidemiologically unrelated to our case, was performed using a standardized pulsed-field gel electrophoresis (PFGE) with *XbaI*.[5] Due to lysis of DNA for both isolates during electrophoresis despite thiourea employed at normal concentration (50 mmol/L in electrophoresis buffer), increased thiourea concentrations were used (650 mmol/L in electrophoresis buffer and 850 mmol/L in the gel).[6] The case isolate and bovine isolate displayed a similar PFGE pattern, in contrast to 3 STEC O174:H21 strains which displayed different patterns (Figure 1). Together, these results provide evidence that both the case and milk isolates were identical. Serum samples from the patient were examined for IgM and IgA antibodies to the lipopolysaccharide of 8 major STEC serogroups by line blot immunoassay, as described previously.[7] No antibody response was detected in iterative sera taken between March 30, 2011, and October 12, 2012.

Although most *Escherichia coli* occur as commensal bacteria, certain strains produce toxins that may cause considerable damage to human organisms, as exemplified by the HUS. With respect to STEC-related typical HUS, humans are usually infected by the ingestion of food derived from cattle, but recently there have been major outbreaks after the ingestion of contaminated sprouts. In Germany and France in June 2011, a large number of cases of HUS (825) were reported.[8,9] These were related to STEC O104:H4 in contaminating fenugreek sprouts and caused 50 fatalities. A widespread belief is that typical HUS does not recur. Since the identification of molecular mechanisms involved in the development of atypical HUS (in adults, mostly caused by a defect in the alternate complement pathway), and of thrombocytopenic purpura (caused by deficient activity of ADAMTS-13), there have been no cases of recurrent typical HUS reported in the literature. The reason for that is not immediately obvious, but the incidence of

typical HUS being very low, the probability of being accidentally exposed twice in a lifetime is also extremely low.

Here, we describe the case of an adult patient who developed 2 episodes of HUS a year apart. HUS was due to the ingestion of raw milk from a cow he kept in his own yard in rural Morocco. This animal was the reservoir of an O174:H21 STEC strain. STEC are zoonotic pathogens. They rarely cause disease in animals, but ruminants are recognized as their main natural reservoir.[10] Lactating dairy animals carry STEC in their intestines, excrete them in their feces, which in turn soil the teats as well as the milk during the milking process.[11] We confirmed that the milk contained the STEC that was the cause of infection. Both STEC isolates (i) belonged to a rare serotype, O174:H21, (ii) had a similar virulence profile, in particular, they contained the *saa* gene rather than the classical *eae* adhesin gene, (iii) had the same multilocus sequence typing type, ST677 (already associated with an O174:H21 STEC isolated in Germany in 2000),[12] and had a similar PFGE profile, which was different from those of 3 other cases of O174:H21, isolated from patients in France during the same year. Despite being uncommon, O174:H21 STEC have been identified as agents causing diarrhea, including bloody diarrhea, and even HUS in patients from different countries.[13] The *eae*-negative STEC strains are more diverse in their relation to animal hosts than typical (*eae*-positive) STEC and some are more specifically associated with sheep or cows. It is known that O174:H21 STEC strains are associated with cattle (http://www.microbionet.com.au/vtectable.htm) and have been isolated from food, mainly from meat (beef and game),[14,15] but also from raw milk and from semihard cow's milk cheeses.[16,17]

Here, the STEC strain could not be isolated from the cow feces, an unsurprising result since fecal shedding of STEC is transient in cattle.[18] Accordingly, because feces from "healthy" cattle are undoubtedly a major source of milk contamination by STEC,[19] and milk from this animal was occasionally distributed locally, thereby posing a serious threat for the whole village, this particular cow was destroyed. Contamination by human feces due to poor hygiene during milking should not be ruled out, however, since humans have been known to be carriers.[20]

Although the first episode of TMA, 6 months before the recurrence, was not microbiologically documented because the patient had no diarrhea (which happened in only 3% of typical HUS cases identified between 1996 and 2006 in France according to the French Institute for Public Health Surveillance), both episodes had occurred shortly after the patient had returned from Morocco where he would drink raw milk from his cow. The absence of systemic antibody response further suggests that

the syndrome was not caused by a common STEC serogroup, such as O26, O55, O91, O103, O111, O128, O145, and O157, which are prevalent in metropolitan France.

## ACKNOWLEDGMENTS

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