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Four cases of hemolytic uremic syndrome – source contaminated swimming water?

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Abstract. In June '93, 4 children, aged 1.5–3.5 years, all living in one town, were admitted to our hospital with the diagnosis hemolytic uremic syndrome (HUS) within one week. In cooperation with the local health authorities a common source was searched for. Questionnaires indicated that the single condition shared by all patients was swimming water. The patients were not acquainted, visited different daycares, and had no food resources in common. All 4 patients bathed in the same, shallow, recreational lake within a period of 5 days. During this time the air temperature was high according to Dutch standards (around 27° C), and many people visited the lake, estimated several hundreds a day. The water level was lower than normal. Diarrhea followed 3–11 days after swimming and the first clinical symptoms of HUS developed 6–7 days after the onset of diarrhea. The lake was closed for swimming when the fourth HUS patient was diagnosed and the possibility of transmission by way of the lake was mentioned. E. coli O157:H7 was demonstrated in the fecal samples of 2 index patients. The samples were taken 9–20 days after the start of diarrhea. Antibodies to O157 and verotoxin 2 were strongly positive in all patients. A local outbreak of diarrheal illness was not registered. Of 16 family members who also swam in the same lake, 7 developed symptoms of enteritis, 3 had positive cultures of their fecal samples and 5 had positive serology. Pulsed-field gel electrophoresis of the E. coli isolates of the patients and family members showed an identical pattern. No O157:H7-DNA could be detected in filter concentrated lake water samples using polymerase chain reaction (PCR) enhancement. These samples were, however, taken 16 days after the latest possible date of contamination of our patients, 15 days after decrease of the air temperature to 15–17° C, and 14 days after the inlet from water from the environment. It could thus very well be that the microorganism was no longer present. This third report of swimming water associated HUS should direct environmental surveys in similar cases of local HUS outbreaks.

Key words: hemolytic uremic syndrome – swimming associated cases – contaminated water – E. coli O157: H7

Introduction

Hemolytic uremic syndrome is clinically characterized by hemolytic anemia, thrombocytopenia and acute renal failure. In its "typical" or infectious form it is preceded by a gastrointestinal syndrome, consisting of mild or bloody diarrhea, vomiting, with or without low grade fever. In children it is usually caused by a verocytotoxin producing enterohemorrhagic E. coli, of which a frequently encountered serotype in our country is O157:H7 [Chart et al. 1991]. Most cases of HUS are sporadic. A number of outbreaks of infection with E. coli O157:H7 are reported, generally transmitted by beef or raw milk [Griffin and Tauxe 1991]. The person to person transmission, by a fecal-oral route, is very easy especially in small children. Infected children under the age of six have a 5% risk of developing HUS [Griffin and Tauxe 1991]. The incidence of HUS in The Netherlands we estimate to be 20 to 30 cases a year, of which 4 to 6 in our hospital, with the highest frequency during the summer.

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Abbreviations: HUS = hemolytic uremic syndrome, PCR = polymerase chain reaction, VT1 = verocytotoxin type 1, VT2 = verocytotoxin type 2, VT2EC = VT2 producing E. coli.

Here we present four cases of HUS, which may have been infected by swimming in possibly contaminated water.

Patients

Four patients, all girls from $1^1/_2$ to $3^1/_2$ years old, were referred to our hospital between June 17 and 23 in 1993 with a hemolytic uremic syndrome (Figure 1). All patients recovered completely.

Case 1 (JS) was $3^1/_2$ years old when she developed abdominal cramps, diarrhea (not bloody) and vomiting on June 11. After 6 days she was referred to our hospital with a hemolytic uremic syndrome. On examination she was pale, drowsy, somewhat edematous with a blood pressure of 135/70 mmHg. She had a few petechiae on the legs. She was oliguric. Laboratory examination revealed an Hb of 4.4 mmol/l with positive parameters of hemolysis (fragmentocytes, high reticulocytes, very high LDH, low haptoglobin), a thrombocytopenia (34×10^9/l), leukocytes (15×10^9/l), renal failure (urea 41 mmol/l, creatinine 313 µmol/l) and a corrected metabolic acidosis (pH 7.37, pCO_2 3.9 kPa, BE –6.0 mmol/l). Treatment was conservative. The acidosis was corrected, protein and fluid restriction were maintained. The oliguria lasted for 24 hours. She needed one red cell transfusion. After correction of the overhydration her blood pressure remained normal.

Case 2 (JO), $1^1/_2$ years old, had diarrhea (not bloody) followed by nausea, vomiting and low grade fever from June 12. Though the gastrointestinal symptoms subsided, she didn't want to eat or drink and appeared ill to the parents. A few petechiae were seen on her abdomen. She remained oliguric. On June 20 a hemolytic uremic syndrome was suspected and she was referred to our hospital. On examination she did not look very ill, had slight generalized edema, a temperature of 39° C, a blood pressure of 115/65 mmHg.

Her laboratory values showed a hemolytic anemia (Hb 5.5 mmol/l), thrombocytopenia (54×10^9/l), slight leukocytosis (11.8×10^9/l), hyponatremia (127 mmol/l), renal failure (urea 33 mmol/l, creatinine 334 µmol/l) and a respiratory corrected metabolic acidosis (pH 7.37, pCO_2 3.6 kPa, BE –7,9 mmol/l). Because of persisting anuria (7 days) and developing hypertension peritoneal dialysis was performed from June 22 until June 29. The ongoing hemolysis necessitated two red cell transfusions and one thrombocyte transfusion before introducing a central venous line and the PD catheter. The hypertension normalized on ultrafiltration.

Case 3 (AB) was a $3^1/_2$-year-old girl who on June 16 developed slimy diarrhea without blood, later followed by vomiting. She had no fever. The defecation pattern normalized but she did not fully recover and her micturition remained poor. The consulted pediatrician suspected HUS and referred her to us. On examination she didn't look ill, was pale, normally hydrated. Blood pressure was normal, temperature 38,5° C, without a focus in upper or lower airways. Laboratory values: Hb 4.7 mmol/l, fragmentocytes positive, reticulocytes 29‰, platelets 26×10^9/l, leukocytes 11.7×10^9/l, CRP 6 mg/l, urea 26 mmol/l, creatinine 168 µmol/l, LDH 2199 U/l, low haptoglobin, pH 7.38, pCO_2 3.8 kPa, BE –6.1. mmol/l.

She remained anuric for 24 hours, after which her diuresis quickly normalized. Her blood pressure remained normal. She received one transfusion of packed red cells. A second bout of fever occurred while no infection focus was found and resolved spontaneously.

Case 4 (NB) was a 3-year-old girl with abdominal cramps since June 9, complicated by bloody diarrhea, nausea, vomiting and fever since June 17. Her condition deteriorated, she became anuric and did not want to drink or eat. On June 23 she was somnolent and showed jittery limb movements. On admission in our hospital she was subcomatose, badly circulated and had serious signs of dehydration. blood pressure 110/70 mmHg, temp. 37.5° C, no signs of meningism, no petechiae.

Laboratory values: Hb 4.7 mmol/l with positive fragmentocytes, platelets 12×10^9/l, leukocytes 26×10^9/l, Na 116 mmol/l, K 4.3 mmol/l, urea 43 mmol/l, creatinine 310 mmol/l, ASAT 131 U/l, ALAT 64 U/l, LDH 6760 U/l, uric acid 1.14 mmol/l, albumin 19 g/l, pH 7.43, pCO_2 5.2 kPa, BE 2.2 mmol/l.

HUS was diagnosed. After careful rehydration and sodium and albumin suppletion her neurological symptoms disappeared, with which a cerebral HUS was excluded. Anuria lasted for less than 24 hours. After June 28 she was polyuric for a few days, suggestive of superimposed acute tubular necrosis. Her decreasing hemoglobin level necessitated several blood transfusions; she received one thrombocyte transfusion prior to insertion of a central venous line. The blood pressure remained normal. The reconvalescent phase was complicated by a *Staph. aureus* sepsis from her arterial catheter. Her renal function recovered completely within a few weeks.

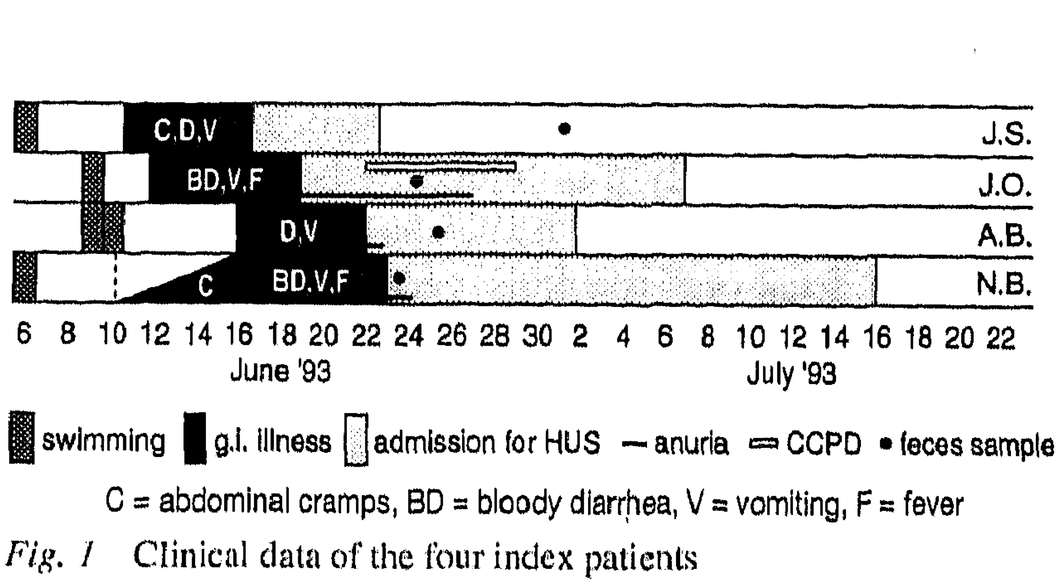

Fig. 1 Clinical data of the four index patients

Epidemiologic investigation

History: A questionnaire was sent to the families, with emphasis on the shops where food, esp. meat, were purchased, restaurant visits, and schools. The four girls live in different parts of the same town. The families were not acquainted. They didn't share schools or butchers, nor had anyone of them drunk raw milk. They had not visited restaurants in the weeks before the illness. The index patients had two features in common: first they all ate ice-creams from the same restaurant, and second they all swam in a semi-natural swimming pool 3 to 11 days before the onset of the diarrhea. In addition to the index patients diarrhea was noted in several family members (Table 1): 2 of the parents, one adult babysitter and 4 of the 8 sibs including one cousin.

A local outbreak of patients with diarrhea was not registered by the local general physicians.

Bacteriology (Table 1). Stool samples from index patients, parents (except the father from case 4), one babysitter and one cousin who visited the swimming pool as well, were precultured on sorbitol McConkey agar to isolate E. coli O157:H7. With antibodies to O157 the presence of E. coli O157 was confirmed. The presence of free verocytotoxin in feces and the E. coli cultures was examined in vero cell-lines. In all investigated persons infection with Salmonella, Shigella, Campylobacter and Yersinia was excluded. Cultures of feces of the index patients were negative in case 1 and case 3; the feces of the two most ill cases (2 and 4) were strongly positive for E. coli O157 and VT2EC; free verotoxin was not shown in both patients. The date of the stool samples are shown in Figure 1. Of the 4 sibs with gastrointestinal illness 2 showed positive bacteriological data; of the 4 without symptoms one had a positive stool culture with E. coli O157:H7. The feces of one parent showed growth of E. coli O157:H7 too.

Water samples from different corners of the lake were taken on June 25. These were cultured under various enriching circumstances. All cultures remained negative. Also samples of silt didn't reveal any E. coli. Samples taken from meat from the butchers of the different families showed negative cultures. Culture of feces of cattle (cows and horses), taken on July 7, remained negative for E. coli O157:H7.

Serology (Table 1). From almost all index patients and family members one or two serum samples were examined for antibodies to the O157 and H7 antigens by agglutination, and to verocytotoxin types 1 and 2 by ELISA.

All index patients showed positive antibody response to O157 and VT2 6 to 11 days after onset of diarrhea. Parents of the index patients did not show a positive antibody titer. The babysitter who joined case 1 with her swim, showed a high titer of antibody to VT2. Of the 4 sibs with diarrhea three were serologically positive.

To test the specificity of the antibody tests a control group was formed: 37 pediatric patients, aged 1 to 6 years, with a disease other than a gastrointestinal or infectious one. A cutoff point of the agglutination test of 1:320 for O157 and 1:160 for H7, and of the ELISA of an optical density of 0.250 was used. The specificity thus reached was 100% for O157, 100% for H7, 94,5% for VT1 and 97.5% for VT2. We then applied these cutoff points to our patients.

Summarizing clinical, bacteriological and serological data (Table 1): All index patients had positive serology and/or bacteriology. Of the adults one parent with diarrhea showed a positive culture, without serologic evidence of disease; case 1's babysitter only showed a positive antibody titer to VT2. Four out of 8 related children had clinical signs, of whom 2 had positive cultures and 2 were serologically positive (1 was positive in both). Of the 4 sibs without clinical signs one showed a positive culture, another a strong antibody response to the H7 antigene.

Polymerase chain reaction (PCR): On all stool samples of patients and family members PCR tests were done to show genes for VT1 and 2, and attaching and effacing factor (EAE). PCR on E. coli O157:H7 from positive stools were positive for VT2 and EAE genes.

The cow dung contained E. coli with expression of either the VT2 or the EAE gene, but not of the two genes simultaneously. The water and silt samples showed E. coli colonies on culture, that were negative in the PCR for VT2 and EAE genes.

In conclusion: the E. coli O157:H7 demonstrated in the patients, could not be demonstrated in the environment.

Table 1 Clinical and laboratory data of index patients and their relatives. C = cramps, BD = bloody diarrhea, V = vomiting, F = fever, **Bold** = index patients

	Clin. signs	Bact.	Serol.	Bact./serol.
J. S.	**C, D, V**	–	+	+
P	–	–	–	–
M	–	–	–	–
Babysitter	–	–	+	+
J. O.	**BD, V, F**	+	+	+
P	–	–	–	–
M	–	–	–	–
F6Y	D, V, F	–	+	+
F10Y	–	–	–	–
F11Y	–	+	–	+
A. B.	**D, V**	–	+	+
P	–	–	–	–
M	–	–	–	–
F11Y	–	–	+	+
S15Y	–	–	–	–
N. B.	**C, BD, V, F**	+	+	+
P	D	n.d.	–	–
M	D	+	–	+
F5Y	D	+	–	+
S7Y	D, V, F	–	+	+
Cousin 10Y	BD, V, F	+	+	+

Discussion

Four children with HUS, all living in one town, admitted within one week to our hospital, all caused by the same E. coli O157:H7, made us suspicious of a single source of infection. There were two common historic features; they all had eaten ice-cream from the same restaurant and they had swum in the same lake. The ice-cream was prepacked and came from a large Dutch factory distributing their products all over the country. If the ice-cream contained E. coli it would have caused a more widespread epidemic instead of this small local accumulation of cases.

The swimming water seemed a more plausible explanation. It is a seminatural, shallow lake. The water is derived from the ditches around it, which are connected to the larger water collections of the Rhine-delta. The ditches drain their water from meadows with cattle, especially cows. Usually the connections of these ditches to the lake are closed, and only opened when the water level of the lake gets too low. The water of the lake is not treated antiseptically, and there is no current in the water. Especially families with small children visit the lake because it is shallow and there are sandy beaches and playgrounds.

Table 2 Reports of water-borne outbreaks of infection with E. coli O157H7

1st author	Country	Year	Source	Source confirmed by culture	No. of pts with GE	HUS
Dev '91	Scotland	'90	drinking water	no	4	0
Swerdlow '92	USA	'90	drinking water	no	243	2
Isaacson '93	South Afrika + Swaziland	'92	surface water	yes	thousands	some
Akashi '94	Japan	'90	drinking water	yes	121	20
Keene '94	USA	'91	surface water	no	24	3

In these June days the weather was exceptionally warm according to Dutch standards (25–28° C). The water level was low and the number of visitors high, several hundreds a day. Contamination may have happened by way of human feces, leftovers of barbecue meat, or cattle dung. The third possibility is very unlikely, since there were no water inlets until the evening of June 9, after all patients had swum at least one time.

The cultured water samples did not reveal the E. coli O157:H7. We must realize that the samples have been taken 16 days after the last opportunity of the children to swim, 15 days after a significant decrease of the air temperature by about 10° C, and 14 days after the dilution of the water by multiple water inlets to increase the water level. These factors may have lowered the density of the microorganisms to an undetectable level.

Five reports [Dev et al. 1991, Swerdlow et al. 1992, Akashi et al. 1994, Keene et al. 1994, Isaacson et al. 1993] have been published on water-borne infections with E. coli O157:H7, as far as we know (Table 2). Three reports concern drinking water, two surface water. In only two of these studies the source was confirmed by culture of the water. In the publications by Dev et al. [1991] and Keene et al. [1994] the E. coli O157:H7 was not cultured, but the water yielded a combination of other fecal microorganisms. One reason for not culturing the specific E. coli could be the delay between infection of the patients and the collection of the water samples. In an experimental situation E. coli O157:H7 survived in water of 20° C for 1 week, after which it declined to zero in about 35 days [Rice et al. 1992].

If the lake was the source of infection, one would expect an outbreak of diarrheal illness. With 4 children with HUS one would expect about a hundred patients with diarrhea [Griffin and Tauxe 1991]. It was not noticed however, by the general physicians. Two factors may contribute to not recognizing a possible outbreak of diarrhea: first, people with diarrhea usually do not visit their doctor, and second, in a town of more than 100,000 inhabitants 100 people with diarrhea may well be missed.

The period of 3 to 11 days that passed between swimming and the occurrence of diarrhea in the index patients is in accordance with the literature. In outbreaks with a known source an incubation period of 3 to 8 days is mentioned [Griffin and Tauxe 1991]. Case 4 had the largest latency of 11 days. This may have been overestimated because during a week before the onset of diarrhea she already complained of abdominal cramps.

It is not strange that the feces samples of two patients remained negative. They were taken 9 (case 3) and 20 (case 1) days after start of the diarrhea. The sample of case 3 was an anal swab. Feces cultures in most patients are negative more than 7 days after the start of the gastrointestinal illness [Tarr et al. 1990].

In the children a stronger tendency to invasion of the E. coli was demonstrated, even though they had mild or no clinical symptoms. From the six family members with diarrhea two were adults. They both didn't show signs of invasion: their serological data were negative. Three of the four children with diarrhea showed positive signs of invasion by positive serology.

In conclusion, four female patients from one Dutch town, aged 1.5–3.5 years, were admitted to our hospital with HUS within one week. E. coli O157:H7 as causative microorganism was proven in all patients. Six of their family members developed gastrointestinal symptoms as well; eight out of the 17 household contacts had either bacteriological or serological proof of contamination with E. coli O157:H7. It is likely that a recreational lake was the source of contamination; unfortunately we could not demonstrate the presence of the E. coli in the environment.

This report indicates that the causative organism of HUS in children can be transmitted through recreational contact with contaminated water. This finding is in agreement with five other reports. We suggest that bacteriological screening of recreational lakes should include tests for the presence of E. coli O157:H7.

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