Haemolytic anaemia after childhood *Escherichia coli* O 157.H7 infection: are females at increased risk?

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SUMMARY

We conducted a 4-year retrospective cohort study to better define the risk of haemolytic anaemia and haemolytic uraemic syndrome (HUS) in children following sporadic gastrointestinal infection with the O 157.H7 serotype of *Escherichia coli*. Of the 72 children infected with this organism, 9 (12·5%) developed haemolytic anaemia, 6 of whom had HUS. No child in a cohort of 72 age-matched controls with *Campylobacter jejuni* gastroenteritis developed haemolytic anaemia ($P = 0·003$). Females had a significantly greater risk of developing haemolytic anaemia after *E. coli* O 157.H7 infection than did males (8/29 females *v.* 1/43 males; $P = 0·003$). In a logistic regression model, female gender emerged as the only statistically significant risk factor for haemolytic anaemia (odds ratio 3·85; 95% confidence interval 1·24–12). These results are consistent with recent reports of a moderate increase in the risk of HUS for females.

INTRODUCTION

Infection with *Escherichia coli* O 157.H7 is strongly associated with the development of the haemolytic uraemic syndrome (HUS) [1], a condition characterized by laboratory findings of haemolytic anaemia, thrombocytopenia, and evidence of acute renal injury [2]. Consistent with earlier reports identifying a wide spectrum of severity in HUS [3, 4], Neill and colleagues have shown that *E. coli* O 157.H7 gastroenteritis is also associated with a subclinical form of HUS, in which there is no obvious reduction in renal function despite the presence of haemolytic anaemia and thrombocytopenia [5].

E. coli O 157.H7 now appears to be the predominant pathogen associated with HUS in many areas [6–9]. The proportion of children who develop HUS following infection with this organism has been reported as 3–26% [7, 10–12], but few studies have included details on the risk of less severe degrees of haemolytic anaemia. Better data on the magnitude of the risk of haemolytic anaemia would be

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desirable, first in determining the frequency with which investigations should be performed in children with *E. coli* O 157.H7 gastroenteritis, and second in defining high risk groups for more intensive study. These data would also be essential for estimating the sample size needed for clinical trials in the secondary prevention of HUS.

The objective of this 4-year retrospective cohort study was to better define the proportion with haemolytic complications among a representative group of children presenting to hospital with *E. coli* O 157.H7 gastroenteritis. A cohort of age-matched children with *Campylobacter jejuni* gastroenteritis was assembled for comparison. The regionalization of inpatient paediatric services in the Ottawa area made it unlikely that children in either cohort whose symptoms worsened would seek further care at another institution.

METHODS

Setting. The Children's Hospital of Eastern Ontario is the only hospital in Ottawa to which children can be admitted. Two general hospitals in adjacent Western Quebec have secondary care paediatric wards, but no children were discharged from either hospital with a diagnosis of HUS (International Classification of Diseases, 9th Revision code 283.10) during the study period.

Patients. Study subjects were identified from the records of culture results in the microbiology laboratory of the Children's Hospital of Eastern Ontario. Eligible patients were children from birth to 18 years in whom *E. coli* O 157.H7 was cultured from stool specimens submitted to the laboratory between 1 January 1985 and 31 December 1988. The laboratory records contained no information on the clinical status of patients. We reviewed the hospital charts of eligible children for presenting symptoms, results of laboratory investigations, and clinical outcomes. One patient transferred to this institution with a diagnosis of HUS was excluded from the study.

Controls. A control group of 72 children whose stool cultures grew *Campylobacter jejuni* was selected from the same laboratory records. Controls were matched for age (± 12 months) and gender, and were selected (without knowledge of their clinical course) from approximately the same time period as the patients with *E. coli* O 157.H7 infection (between February 1984 and February 1989).

Clinical definitions. Haemolytic anaemia was defined as a haemoglobin value under two standard deviations below the mean for age [13], with evidence of red blood cell (RBC) fragmentation on the peripheral smear, as reported at the time of illness by technicians in the haematology laboratory. Throughout the 4-year study period, RBC haemolysis was scored as: 5 or fewer fragments per 1000 RBCs, non-specific; 6–50 fragments, 1 + ; 51–100 fragments, 2 + ; 101–250 fragments, 3 + ; > 250 fragments, 4 + . Blood smears from patients or controls in whom the haemoglobin was less than 105 g/l were retrieved from storage. A senior haematology laboratory technician confirmed all initial interpretations of the presence or absence of RBC fragmentation.

The haemolytic uraemic syndrome was defined as the acute onset of haemolytic anaemia, thrombocytopenia (platelets $< 150 \times 10^9$/l), and uraemia (urea > 7 mmol/l, the upper limit of the reference range for this laboratory).

Culture techniques. Beginning in January 1985, stool specimens submitted to the microbiology laboratory for bacterial culture were routinely tested for *E. coli* O 157.H7. Following inoculation on a MacConkey agar containing 1% sorbitol, colonies which failed to ferment sorbitol were identified as *E. coli* by standard biochemical tests. Isolates were then serogrouped by agglutination of a boiled suspension, using an O 157 antiserum. All isolates of *E. coli* O 157 were forwarded to the Laboratory Centre for Disease Control (LCDC) Enteric Reference Laboratory for more complete serotyping and verotoxin assay [14]. Detection of verotoxin genes was determined by the polymerase chain reaction [15].

Statistical analysis. Continuous variables were compared using t tests (two-tailed) and categorical variables were compared using the χ^2 with Yates' correction or the Fisher's exact test. The variables predictive of HUS were analysed using stepwise logistic regression (BMDP Statistical Software, Inc., Los Angeles, California). Confidence intervals for proportions were calculated by the method of Daniel [16]; the confidence interval (CI) for the odds ratio from the logistic regression analysis was calculated by the method of Schlesselman [17], and the CI for the relative risk was calculated using the Epi Info software (Version 3, USD, Inc., Stone Mountain, Georgia).

RESULTS

Patients with E. coli *O 157.H7 infection.* Over the 4 years of study, 72 patients infected with *E. coli* O 157.H7 were investigated (43 males, 29 females). In 64 (including all those with haemolytic anaemia) verotoxin 1 and verotoxin 2 genes were detected using the PCR technique. In 4 patients a single gene for verotoxin was detected (3 with verotoxin 2; 1 with verotoxin 1 only – an uncommon pattern). In isolates from 2 siblings no verotoxin activity was detected and in 2 other patients the assay was not performed.

The median age in the study patients was 4 years (range, 3 months–17 years). Cultures were obtained a median of 3 days (range, 1–12 days) after the onset of symptoms; 93% of cultures were obtained within 5 days of the onset of illness. Sixty-four percent of patients presented during the summer months (June–August); 32 children (44%) were admitted to hospital. The median length of hospital stay was 4 days (range, 1–28). Hospital records were available for all but the three children whose cultures were forwarded to the laboratory from physicians' offices. Of the 69 in whom a record of presenting symptoms was available, 92% had bloody diarrhoea. Four patients each had one other possible intestinal pathogen identified (adenovirus, *Clostridium difficile* toxin, *Giardia lamblia*, and *Salmonella saint-paul*).

Complications of E. coli *O 157.H7 infection.* One patient developed rectal prolapse; one had an ileocolic intussusception reduced with barium; and two patients underwent laparotomy and appendectomy as part of the investigation of their abdominal pain. Five patients with haemolytic anaemia received transfusions of packed red blood cells and two received peritoneal dialysis for 10 and 25 days respectively. There were no deaths in this cohort; no patients required peritoneal dialysis after discharge.

Proportions with haemolytic anaemia. The table presents the pertinent clinical

and laboratory variables on the nine patients (12·5%) in this cohort who developed haemolytic anaemia (95%, CI, 5–20%). One other patient, a 3-month-old female, had RBC fragments on the blood smear, but her lowest measured haemoglobin was 102 g/l, a value within two standard deviations of the mean for age. Of the nine with haemolytic anaemia, six satisfied the study definition of HUS. With the exception of a 1-year-old girl with *E. coli* O 157.H7 infection who had been transferred from another hospital with a diagnosis of HUS, and therefore was excluded from the study, no other patients were admitted to this institution with HUS in the 4-year study period. A search of the Canadian Pediatric Kidney Disease Reference Centre database revealed that none of the patients in this cohort had been admitted with HUS to other children's hospitals in Canada during the period from January 1986 to December 1988 [18].

Red cell fragmentation was noted a median of 8 days after the onset of gastrointestinal symptoms (range, 3–14 days). In only 3 of the 9 patients with haemolytic anaemia were RBC fragments evident on the day of admission.

Risk factors for haemolytic anaemia. The difference in the proportion of males and females who developed haemolytic anaemia was statistically significant (1/43 *v.* 8/29; $P = 0·003$). The relative risk of developing haemolytic anaemia for females with *E. coli* O 157.H7 infection in this cohort was 11·9, although the 95% confidence interval (1·2–71) was wide. When only those less than 5 years of age were analysed, the effect of gender on the risk of haemolytic anaemia remained statistically significant (7/18 *v.* 1/19; $P = 0·04$, Fisher's exact test). Overall, there was no difference between females and males with regard to potential confounding variables such as age (mean ± S.D., 5·5 ± 4·7 *v.* 6·9 ± 4.8; $P = 0·24$), the proportion admitted to hospital (52 *v.* 40%; $P = 0·31$), or the number of days of symptoms before the stool culture was obtained (3·3 ± 2·5 *v.* 3·2 ± 1.9; $P = 0·85$). More females (20/29) than males (22/43) had at least one haemoglobin determination ($P = 0·07$); among those with at least one haemoglobin determination, the proportion of females with haemolytic anaemia remained statistically significant (8/20 *v.* 1/22; $P = 0·008$, Fisher's exact test). Ascertainment of haemolytic anaemia was incomplete since laboratory tests were ordered at the discretion of the attending physicians. In 30 patients (42%) no blood samples were obtained, but none of these children were considered sick enough to require admission to hospital. When the variables age, sex, admission to hospital, and the number of blood tests were entered into a logistic regression model, the only significant risk factor for haemolytic anaemia was female gender, with an odds ratio of 3·85 ($P < 0·001$; 95% confidence interval, 1·24–12).

In the week before the stool culture was obtained, 7 patients (4 of whom developed haemolytic anaemia) received antiemetic or antidiarrhoeal medications, and 7 patients (3 of whom developed haemolytic anaemia) received antibiotics. The proportion of patients with first white blood cell values greater than $15 \times 10^9/l$ was higher among those who developed haemolytic anaemia (56 *v.* 14%, $P = 0·02$).

Controls. There was no significant difference between the *E. coli* patients and the controls with regard to the proportion admitted to hospital (44 *v.* 49%; $P = 0·62$), proportion with two or more haemoglobin determinations (36 *v.* 40%; $P = 0·61$), or the proportion with two or more urinalyses (46 *v.* 40%; $P = 0·87$). Although six

Table 1. *Clinical and laboratory features in nine patients with HUS or haemolytic anaemia after* E. coli O 157.H7 *infection*

| | Age (yr) | Sex | Values on presentation | | | Low hgb (g/l) | Low Plt ($\times 10^9$/l) | Peak urea (mmol/l) | RBC fragments (1+–4+) |
			Hgb (g/l)	Plt ($\times 10^9$/l)	Urea (mmol/l)				
1.	2	M	69	58	19·3	61	58	20	2+
2.	2·5	F	123	63	9·1	62	30	25·1	1+
3.	8	F	140	414	3·1	55	64	17·7	3+
4.	12	F	152	289	4·1	89	14	30·5	3+
5.	3	F	151	113	21·3	78	72	25·5	1+
6.	4	F	135	425	5·1	71	53	19·1	1+
7.	1·8	F	121	426	1·8	105	129	5	1+
8.	1·2	F	101	153	6·1	76	153	6·1	2+
9.	1·3	F	55	449	8·6	55	399	9·8	2+

Abbreviations: hgb, haemoglobin; plt, platelets; RBC, red blood cell; 1+–4+, scoring described in Methods section.

controls had haemoglobin levels below 105 g/l (range, 85–104 g/l), significantly more *E. coli* O 157.H7 patients than controls had haemolytic anaemia (9 *v.* 0; $P = 0·003$), thrombocytopenia (7 *v.* 1; $P = 0·023$), and uraemia (7 *v.* 1; $P = 0·022$).

DISCUSSION

This retrospective cohort study of 72 children presenting to hospital with *E. coli* O 157.H7 gastroenteritis identified nine patients (12·5%) who developed haemolytic anaemia. Six of these nine satisfied study criteria for the diagnosis of HUS. Because no other institutions in the region provide tertiary care to children, we assume there was complete ascertainment of all patients in the cohort who developed symptomatic HUS.

The true risk of haemolytic anaemia for those who present to hospital with colitis due to *E. coli* O 157.H7 may be higher than 12·5%, as haematologic studies were obtained infrequently in children who were not admitted to hospital. The rate of HUS in this study (8%) is similar to that reported among symptomatic children in outbreaks of *E. coli* O 157.H7 gastroenteritis (7–8%) [10, 11]. Since the earlier epidemiologic investigations began after the gastroenteritis outbreaks were established, their primary emphasis was on attack rates of diarrhoea. Laboratory assessment of acute haemolytic anaemia in most patients would not have been possible, thereby leading to an underestimate of the risk of haemolytic complications. Our results differ from another large study of sporadic *E. coli* O 157.H7 infection in childhood, in which 26% (9/34) developed HUS, but this investigation included patients in whom the diagnosis of HUS had been made at another hospital before referral [7]. We deliberately excluded such patients in order to arrive at a more generalizable estimate of risk.

The children in our study likely represent the more serious end of the clinical spectrum of *E. coli* O 157.H7 gastroenteritis, as over 90% had bloody diarrhoea. As was noted in the study by Pai and colleagues [12], infected patients with milder symptoms might not have visited the hospital and, in accordance with current

practice [19], stool specimens from patients with bloody diarrhoea might not have been submitted for culture.

An unexpected observation from these data is that females have a significantly higher relative risk of haemolytic anaemia after *E. coli* O 157.H7 infection than do males. Our results are consistent with a recent study which found male gender to protect against the progression of *E. coli* O 157.H7 colitis to HUS (OR = 0·27; CI 0·083–0·852) [20]. Nonetheless, other large studies of sporadic childhood *E. coli* O 157.H7 gastroenteritis have not identified gender as a risk factor for haemolytic anaemia [12], and our results await confirmation in a prospective study with a higher rate of haematologic investigations.

If females indeed are at an increased risk of haemolytic anaemia after *E. coli* O 157.H7 infection, one would expect this to be reflected in an increased female-to-male ratio in HUS. In the era before verotoxin-producing *E. coli* had been identified, HUS had been reported to have an equal gender ratio [2, 21]. However, more recent population-based studies in Oregon from 1979–82 and in the Baltimore–Washington area from 1979–83 found female-to-female ratios of 1·3 and 4·0, respectively, for children hospitalized with HUS [4, 22]. Voluntary reporting of HUS in Britain in 1983–4 identified 71 patients, of whom 40 were female (female-to-male ratio 1·3) [23]. Of patients reported by participating nephrologists to the Canadian HUS registry between 1986–8, 126 of 226 were female (female-to-male ratio of 1·26) [18].

The mechanism of an increased risk of haemolytic anaemia among females remains to be determined. Verotoxins, or Shiga-like toxins, are strongly associated with the development of HUS, although their role in the pathogenesis of the clinical syndrome is unclear [24, 25]. We are aware of no data which suggest differences between males and females in exposure to verotoxin-producing organisms, in the size of the inoculum or the adherence of verotoxigenic *E. coli* to intestinal epithelium, in the concentration of verotoxin to which the renal endothelium is exposed, or in the density of verotoxin receptors in the kidney or on red blood cells [26–28].

We hypothesize instead that a difference between males and females in the risk of haemolytic anaemia after *E. coli* O 157.H7 infection is related to difference in host response. It has been appreciated for some time that the immunoregulatory genes on the X-chromosome confer both an advantage for females in combating infection [29, 30], and a disadvantage in predisposing them to autoimmune disease [31]. Girls have higher concentrations of specific antibodies to *E. coli* antigens than boys [32, 33]; IgM concentrations in females exceed those in males by a factor of 30% [34], and serum IgM concentrations correlate with the number of X chromosomes [35]. The onset of haemolytic anaemia after *E. coli* O 157.H7 infection, characteristically at the end of the first week of the illness, is consistent with the time required for the development of a primary IgM antibody response. If Leung and colleagues are correct in suggesting that the production of anti-endothelial cell antibodies is important for the pathogenesis of vascular damage in HUS [36], then it is reasonable to speculate that females are more likely to develop the condition because they are capable of a more vigorous anti-endothelial cell antibody response. This hypothesis could be tested in a prospective study of patients with *E. coli* O 157.H7 gastroenteritis.

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