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Diarrhoea in close contacts as a risk factor for childhood haemolytic uraemic syndrome

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SUMMARY

To determine whether the risk factors for childhood haemolytic uraemic syndrome (HUS) are similar to risk factors previously reported for *Escherichia coli* O 157.H7 gastroenteritis, we conducted a case-control study at eight paediatric hospitals in the summer of 1990. Thirty-four consecutive children with HUS were prospectively enrolled; all had diarrhoea and 88% had laboratory evidence of exposure to verotoxin-producing *E. coli* (VTEC). The 102 controls were otherwise healthy children with minor acute injuries. Parents of all subjects responded to a questionnaire about each child's exposure to various foods, methods of food preparation, sources of water, travel, and individuals with diarrhoea.

Children with HUS were significantly more likely than controls to have had close contact with an individual with diarrhoea in the 2 weeks before the onset of illness (74 *v.* 29%, $P < 0{\cdot}00001$; odds ratio 7·0, 95% CI 2·7–18·5). The onset of diarrhoea in the contacts occurred a median of 6 days (range, 1– > 14 days) before the onset of diarrhoea in the HUS patients. Exposure to undercooked ground meat was not significantly more common in the patients with HUS (15 *v.* 8%; $P = 0{\cdot}05$). These data provide evidence consistent with person-to-person transmission of VTEC in a substantial proportion of episodes of childhood HUS.

INTRODUCTION

The haemolytic uraemic syndrome (HUS), a leading cause of both acute and chronic renal impairment in childhood, most commonly affects previously healthy children in the week following the development of a gastrointestinal illness [1]. It is now clear that the gastrointestinal pathogen most frequently isolated from North American and European children with HUS is *Escherichia coli* O 157.H7 [2–6]. Exposure to undercooked ground beef is the predominant risk factor identified in both epidemics and sporadic instances of *E. coli* O 157.H7 [7–14], although outbreaks have been reported after ingestion of unpasteurized milk, cheese, improperly refrigerated cold cuts, exposure to contaminated water, and through person-to-person spread [15–17].

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Little information is available regarding whether the risk factors for sporadic childhood HUS are similar to those reported for *E. coli* O 157.H7 gastroenteritis. Because such information is valuable for the development of strategies to prevent HUS, we initiated a multicentre case-control study of the issue in children with the disease. Contact with individuals with diarrhoea, rather than direct exposure to undercooked meat, emerged as the most important risk factor for childhood HUS.

METHODS

The study was conducted during the summer of 1990 at the eight hospitals listed in the Appendix. From 1 May 1990 to 31 August 1990, patients less than 15 years of age were eligible for participation if they had been diagnosed as having HUS by a paediatrician or paediatric nephrologist, and if that diagnosis was validated by the following laboratory criteria: (*a*) haemolytic anaemia of recent onset (haemoglobin concentration under two standard deviations below the mean for age, with evidence of schistocytes in the peripheral blood; (*b*) creatinine concentration > 97th percentile for age or urea > 7 mmol/l (20 mg/dl). Thirty-four consecutive patients with HUS were enrolled in the study; 26 of these had *E. coli* O 157.H7 infection, 2 had other verotoxin-producing *E. coli* serotypes, and 2 had serological responses to verotoxin but no verotoxigenic *E. coli* isolated on culture of the stools [19].

At each institution, otherwise healthy controls were recruited from the emergency department roster of children with minor injuries. These children were selected as controls because they were unlikely to have modified their food intakes for medical reasons in the preceding 2 weeks. Controls were excluded if they had chronic illnesses, gastrointestinal illness, or food allergies, and if the risk factor questionnaire was administered more than 7 days after HUS had been diagnosed in the matched case.

We attempted to match four controls by age (±1 year) and gender for each HUS patient whose stool culture grew *E. coli* O 157.H7. Two healthy controls were not obtained early in the study, resulting in 102 rather than 104 controls for the 26 patients with HUS and *E. coli* O 157.H7. In preliminary analyses, the other 8 HUS patients (without *E. coli* O 157.H7 on stool culture) had similar clinical and epidemiological features to the 26 with *E. coli* O 157.H7 infection. The two groups with HUS were therefore collapsed into one, and were compared in a conservative unmatched analysis to the 102 controls. This resulted in 3 controls for each of the 34 HUS patients.

A research assistant in each hospital administered a 45-minute risk factor questionnaire to the parents of all HUS cases and controls. The parent of the child with HUS was interviewed face-to-face. Parents of controls were interviewed by telephone. The time period to which most questions referred was the 14 days before the onset of illness in the HUS patient, and the 14 days before the control child was evaluated in the emergency department.

The risk factor questionnaire was designed for this study, and was pilot tested for content validity by physicians and nurses. Parents were asked about the child's exposure to household and non-family contacts with diarrhoea, recent travel, visits to a farm, contact with animals, sources of water, and exposures to

various foods. If the child had eaten meat in the preceding 2 weeks, the parent was asked whether there was some pink present in the meat, or no pink at all. Items regarding demographic data and questions about usual household food preparations practices were also included in the questionnaire. Parental report was used to determine whether diarrhoea was present in the subjects and their contacts. The study was approved by the research ethics committees of the participating institutions.

Statistical comparisons were conducted using SPSS/PC+V4.0 (Chicago, Illinois). An unmatched analysis of cases and controls was conducted. Continuous variables were compared using two-tailed t tests, and categorical variables were compared using the Chi-square statistic or the Fisher exact test. Because the number of comparisons between groups was large, only differences at the $P < 0{\cdot}01$ level were considered statistically significant. Differences at the $0{\cdot}01 < P < 0{\cdot}05$ level are reported as trends. Odds ratios and their confidence intervals were calculated using the Confidence Interval Analysis software (*British Medical Journal*, 1989). Attributable risk estimates designed for retrospective studies were calculated under the assumption that the incidence of HUS is low and that the control group is representative of the population of interest. Approximate 95% confidence intervals for the attributable risks were calculated based on the method of Walter [20].

RESULTS

All 34 HUS patients had diarrhoea, and in 29 the diarrhoea was bloody. The ages of the 34 HUS patients and the 102 controls were similar (mean ± S.D., $4{\cdot}4 \pm 3{\cdot}9$ *v.* $4{\cdot}3 \pm 3{\cdot}5$ years; $P = 0{\cdot}91$); 62% of HUS patients and 67% of controls were female ($P = 0{\cdot}60$). There was no difference between the groups in the proportion of mothers who had completed high school (62 *v.* 58%; $P = 0{\cdot}84$), but there was a trend to a higher proportion with two or more children less than 5 years of age in families of HUS patients (41 *v.* 22%; $P < 0{\cdot}05$).

In Canada, approximately 24% of patients with HUS live in rural areas [6] and these children are more likely than are children with minor injuries to be referred to paediatric hospitals for treatment. As expected, more HUS patients than controls lived > 25 kilometers from the participating hospital (41 *v.* 11%; $P < 0{\cdot}001$).

The median number of days between admission to hospital and administration of the questionnaire to the parents of HUS patients was 3 days (range 1–22 days). Seventy-five percent were interviewed within a week of admission.

Contact with an individual with diarrhoea, whether inside or outside the household, was significantly more common for HUS patients in the 2 weeks prior to the onset of illness than for controls in the 2 weeks preceding the interview (Table 1). For children less than 5 years of age, contact with an individual with diarrhoea remained a statistically significant risk factor ($P < 0{\cdot}0002$). As illustrated in Fig. 1, the onset of diarrhoea in the contact occurred a median of 6 days before the onset of diarrhoea in the patients with HUS (range, 1–> 14 days).

Parents or children identified a clear exposure to undercooked ground beef in only three instances (one from a hamburger at an outdoor camp where the meat

Table 1. *Hus patients and controls reporting contact with an individual with diarrhoea*

	Percentages						
	HUS	Controls					
Exposure	($n = 34$)	($n = 102$)	P	OR	95% CI	AR	95% CI
Household member with diarrhoea	68	25	< 0·0001	5·9	2·3–15·0	0·57	0·29–0·74
Non-household contact with diarrhoea	26	5	0·001	7·1	1·9–29·0	0·23	0·05–0·37
Any diarrhoea contact	74	29	< 0·00001	7·0	2·7–18·5	0·63	0·34–0·79
Undercooked meat (pink centre)	15	8	0·05	2·0	0·5–7·6	0·07	−0·08–0·20

Abbreviations: OR, odds ratio; CI, confidence interval; AR, attributable risk.

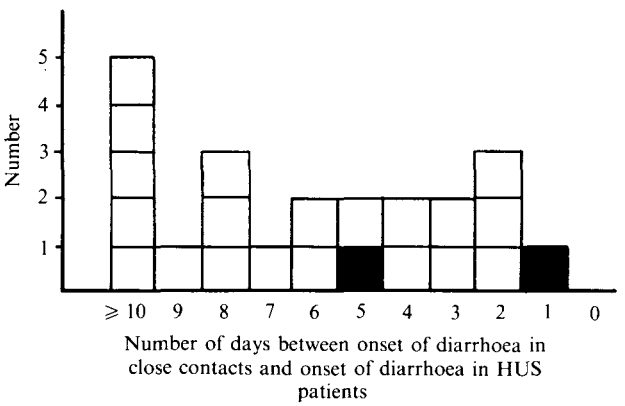

Fig. 1. Onset of diarrhoea in 22 close contacts of HUS patients. Each block represents the time of onset of diarrhoea in a close contact in relation to the onset of diarrhoea in the HUS patient. The solid blocks represent patients in whom there was a clear history of exposure to undercooked ground meat. In three patients not shown, diarrhoea in the close contact preceded diarrhoea in the HUS patient by an undetermined number of days.

was 'almost not cooked at all', one from raw ground beef placed directly into simmering spaghetti sauce and cooked for only 5 min, and one from a foul-tasting hamburger consumed at a soup kitchen). There were no overall differences between groups in the rates of exposure to ground meat, cuts of beef, or eggs. HUS patients were marginally more likely to have eaten ground meat with some pink in the centre (15 *v.* 8%; $P = 0·05$) or to have been exposed to unpasteurized milk (9 *v.* 1%; $P = 0·05$), and were also less likely to have eaten chicken (74 *v.* 91%: $P = 0·02$).

More HUS patients drank water from a well in the 2 weeks before onset of the illness (44 *v.* 19%; $P < 0·01$). There were no differences between groups in the usual methods of defrosting or cooking meat. More HUS patients than controls had visited a farm (38 *v.* 19%; $P < 0·01$), and more reported having contacted with cows (26 *v.* 18%; $P < 0·002$) in the 2 weeks before the illness. When the analysis was restricted to those who lived in an urban area, there was no difference

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between the groups in the proportion who had visited a farm, had contact with cows, or drank unpasteurized milk; however the trend towards a statistically significant difference in exposure to well water remained (36 *v.* 14%; $P = 0{\cdot}03$).

The attributable risk estimates from these data (Table 1) suggest that the rate of HUS could have been reduced by nearly two-thirds if exposure to individuals with diarrhoea could have been avoided. The 95% confidence interval for the attributable risk of exposure to undercooked meat includes zero, and is therefore not statistically significant.

DISCUSSION

In this multicentre case-control study, close contact with an individual with diarrhoea overshadowed direct exposure to undercooked ground meat as a risk factor for the development of sporadic childhood HUS. In the 2 weeks before the onset of gastrointestinal symptoms, the risk of having had contact with an individual with diarrhoea was 7 times higher in HUS patients than in controls ($P < 0{\cdot}00001$). In contrast, rates of exposure to undercooked ground meat did not differ significantly.

The high rate of gastrointestinal symptoms in close contacts of HUS patients could be explained by common exposure to contaminated food or water, by common exposure to a different non-human source of bacterial contamination (e.g. farm animals), or by person-to-person spread of verotoxigenic *E. coli*. In epidemics in which food exposure occurs on a clearly specified day, the median incubation period for *E. coli* O 157.H7 infection in 3–4 days [16, 21]. In the christening party outbreak reported by Salmon and colleagues, all those who developed diarrhoea more than 5 days after the common food exposure had household contact with an individual who had confirmed *E. coli* O 157.H7 infection, consistent with secondary spread of the infection [16]. When the interval between the onset of gastrointestinal symptoms in the contacts and the HUS patients in our study was less than 4 days, simultaneous exposure to contaminated food would be a reasonable explanation for the common symptoms. For those with a longer interval (e.g. $\geqslant 5$ days) between onset of diarrhoea in the contacts and the HUS patients, common exposure to contaminated food would have to be explained either by an improbable difference in the length of the incubation period, or differences in the times that frozen contaminated food was eaten. A more parsimonious explanation is that many of these episodes of gastroenteritis preceding HUS were due to person-to-person spread of VTEC.

Transmission of infection from patients to staff has been suspected in a nursing home epidemic of *E. coli* O 157.H7 gastroenteritis [11], in hospitals [22], and from patients to staff or family members in school outbreaks [13–15, 18], but it has not been considered a primary method of acquiring disease. Person-to-person transmission has been suggested as a possible cause in a family outbreak of HUS [23] and person-to-person spread of other enteric pathogens is well documented [24–27]. In Argentina, where a high proportion of HUS patients have evidence of exposure to verotoxin (Shiga-like toxin) [28] asymptomatic infection of close contacts of children with HUS is common. Among family contacts of 51 children with HUS, free faecal cytotoxin was identified from 39% and antibody titres to Shiga-like toxin were present in 75%, although gastrointestinal symptoms within

the preceding 2 weeks were reported by only 1 of 87 individuals [29]. Lopez and colleagues attribute this low rate of symptoms to a high level of prior immunity in the family contacts. In North America, prior immunity to verotoxigenic *E. coli* may be less common, resulting in a higher proportion of symptomatic individuals among those exposed.

Certain aspects of the study methodology deserve further comment. First, because the main results were somewhat unexpected, our investigation did not include a detailed examination of the food exposure history of the close contact with diarrhoea, nor were stool cultures obtained from these individuals. It would be important to obtain such information in subsequent examinations of the role of person-to-person transmission of VTEC in the development of HUS. Although we did not formally assess its validity, the history of diarrhoea was likely to have been accurate in the 23 close family contacts. Second, the possibility of recall bias must be acknowledged due to the delay in interviewing the parents of some HUS patients. Parents who became aware of the association of HUS with undercooked ground meat may have under-reported their children's exposure to this risk factor. Our experience, however, was that exposure to undercooked meat was reported more frequently during questionnaire administration than in the admission history in the medical chart, suggesting that deliberate under-reporting of such exposures was infrequent.

Third, as is unavoidable in studies of this type, parents may not have been aware of the child's complete food exposure history, although older children with HUS were encouraged to supplement the parent's responses. This could have had the effect of underestimating the risk attributable to undercooked ground meat. Fourth, the different method of questionnaire administration for cases and controls was dictated by convenience, due to the ready availability in hospital of the parents of HUS patients. This may have introduced bias. Thornberry has provided evidence that more health-related events may be reported in telephone interviews than in those conducted face-to-face [30]. If true in our study, the effect of this bias would have been to minimize the differences between the HUS patients and the controls.

Our study provides preliminary evidence that person-to-person acquisition of VTEC may be a more important factor in the development of sporadic childhood HUS than is direct exposure to undercooked ground beef. If confirmed in subsequent studies, this finding has important implications for the prevention of HUS. Assiduous attention to hygienic measures to prevent the spread of VTEC infection within families has the potential to prevent a substantial proportion of HUS episodes in childhood. While encouraging thorough cooking of ground meat served to children is a sensible public health strategy, our results suggest that doing so is unlikely to prevent the majority of HUS cases in the age group at greatest risk, unless the family and general population exposure to VTEC is reduced.

APPENDIX

The investigators of the CPKDRC HUS study are: British Columbia Children's Hospital, Vancouver, British Columbia: James Carter, M.D., Nevio Cimolai, M.D. (principal investigators), David Lirenman, M.D., John Anderson, M.D., Misao

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