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Haemolytic uraemic syndrome and Shiga toxin-producing *Escherichia coli* infection in children in France

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SUMMARY

We conducted a study to determine the incidence of haemolytic uraemic syndrome (HUS) in children in France and to assess the role of Shiga-toxin-producing *Escherichia coli* (STEC) infection in the aetiology of HUS. In collaboration with the Société de Néphrologie Pédiatrique we undertook a retrospective review of all cases of HUS hospitalized from January 1993 to March 1995 and a 1-year prospective study (April 1995–March 1996) of epidemiological and microbiological features of cases of HUS. The polymerase chain reaction (PCR) procedure was used to detect *stx*, *eae*, *e-hlyA* genes directly from case stool samples. Serum samples from cases were examined for antibodies to lipopolysaccharide (LPS) of 26 major STEC serogroups. Two hundred and eighty-six cases were reported. The average incidence per year was $0{\cdot}7/10^5$ children < 15 years and $1{\cdot}8/10^5$ children < 5 years. During the prospective study, 122/130 cases were examined for evidence of STEC infection using PCR and/or serological assays and 105 (86%) had evidence of STEC infection. Serum antibodies to *E. coli* O157 LPS were detected in 79 (67%) cases tested. In conclusion, this study showed that STEC infection is an important cause of HUS in children in France, with a high proportion related to the O157 serogroup.

INTRODUCTION

Haemolytic uraemic syndrome (HUS) and Shiga-toxin-producing *Escherichia coli* (STEC) infections are not reportable in France and until recently, the incidence, aetiology and risk factors of HUS in France were not well known, although some clusters had been reported [1, 2]. In this context, a national study was conducted in 1995 and 1996 to assess the incidence of HUS, to describe the clinical characteristics of patients and to determine the proportion of cases associated with STEC infection.

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MATERIALS AND METHODS

Study design

The study consisted of a retrospective review of all cases of HUS in children under 15 years of age hospitalized in paediatric nephrologic centres from

January 1993 to March 1995 and a 1-year prospective study of the clinical, epidemiological and microbiological features of HUS incident cases under 15 years old hospitalized between April 1995 and March 1996. All the members of the Société de Néphrologie Pédiatrique from the 31 French paediatric nephrology centres participated in the study. In addition, we wrote to all general paediatricians working in public hospitals and asked them to report cases of HUS.

Case definition

A case of HUS was defined as a child under 15 years of age with acute haemolytic anaemia (haemoglobin < 10 g/100 ml with evidence of red cell fragmentation) and acute renal injury defined as a serum creatinine level $> 60\,\mu$mol/l if age < 2 years, $> 70\,\mu$mol/l if age $\geqslant 2$ years, and/or haematuria > 20000/ml and/or proteinuria > 1 g/l.

A case was defined as having STEC infection if STEC strains had been isolated in the stools or if there was evidence of Shiga-toxins (*stx*) genes in stool samples, or if antibodies to *E. coli* lipopolysaccharide (LPS) of 26 serogroups were detected in serum samples.

Data collection

The following data were collected for each patient: demographic information, prodromal diarrhoea, use of antibiotics or antimotility agents, acute complications, treatment of HUS, immediate outcome, other cases of HUS or diarrhoea in the household, day-care centres or school attendance in the 15 days prior to onset and exposure to potential risk factors (unpasteurized dairy products, ground beef, contact with farm animals and swimming activities). Further information was collected only in the prospective study (treatment of diarrhoea, acute complications, exposure to risk factors).

Faecal samples

A faecal swab was obtained from each child, placed in transport medium and sent at room temperature by express mail to the Laboratory of Microbiology, Hôpital Robert Debré, Paris, for immediate processing. It was used to inoculate a tryptocasein soy broth which was incubated during 3 or 4 h. After centrifugation, a part of the sample was frozen and sent to the Unité des Entérobactéries, Institut Pasteur, for further analysis.

All specimens were cultured for bacterial pathogens by established microbiological techniques including plating on sorbitol MacConkey agar, biochemical tests and serotyping using O157 and H7 antisera for detection of *E. coli* O157:H7.

At Hôpital Robert Debré, direct detection of *stx* genes was performed by polymerase chain reaction (PCR) technique as described previously [3]. Bacterial colonies from samples positive by PCR were run in an identical PCR for confirmation and were serotyped.

At Institut Pasteur, three genes were detected by PCR directly: *stx* (all variants) encoding Shiga-Toxin, *eaeA* encoding the outer membrane protein intimin, and EHEC-*hlyA* encoding the EHEC haemolysin [4]. In positive samples, colony blot and Southern hybridizations with *stx* probe were done and all stx-positive isolates were also tested for *stx*, *eaeA*, EHEC-*hlyA* [5–6].

All the positive results whatever the PCR technique performed for the detection of *stx* genes were considered as a positive PCR.

Serology

Serum samples were obtained at the time of diagnosis (D0), 15–20 days later (D15) and, if possible, 6–8 weeks after the onset of HUS (D40) and sent to Institut Pasteur.

Serum samples were examined for antibodies (IgA, IgM, IgG) to *E. coli* LPS from 26 serogroups (O:1, O:2, O:4, O:5, O:9, O:25, O:26, O:29, O:55, O:100, O:103, O:104, O:105, O:111, O:112, O:113, O:115, O:118, O:127, O:128, O:136, O:145, O:153, O:157, O:163, O:164) by line blot immunoassay and the frequency and kinetics of the immune response were analysed [7].

Statistical analysis

To determine overall and age-specific incidence rates of HUS, population estimates were obtained from National Statistics. Data analysis was conducted by using Epi-Info, version 6.01 (CDC, Atlanta). Categorical variables were compared with the χ^2 or Fisher exact test as appropriate.

RESULTS

Epidemiology

Between January 1993 and March 1996, 286 cases of HUS were reported in France (retrospective study 156 cases, prospective study 130 cases). During the

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Table 1. *Annual number of paediatric cases and incidence rate of HUS* (*France, 1993–5*)

Year	Number	Incidence rate/10^5 children per year
1993	58	0·5
1994	94	0·8
1995	94	0·8
1993–95	246	0·7

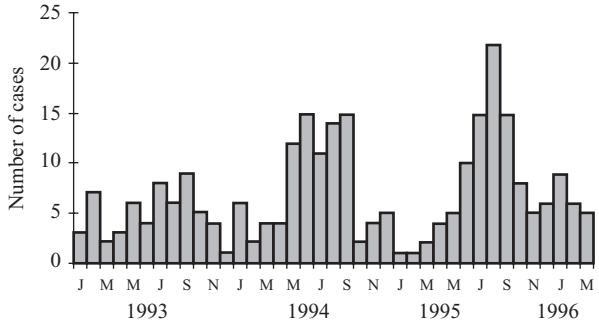

Fig. 1. Cases of HUS in children by month (France, January 1993–March 1996).

Table 2. *Incidence rate of HUS by age group* (*France, 1993–5*)

Age (year)	Number (%)	Mean annual incidence rate/10^5 children
< 2	125 (51)	2·8
2–4	75 (30)	1·1
5–9	31 (13)	0·3
10–14	15 (6)	0·1
Total	246 246	0·7

prospective study (April 1995–March 1996), 113 cases were notified by the paediatric nephrologists and 17 by the general paediatricians. To compare incidence rates by year, 17 cases reported by the general paediatricians in 1995–6 and 3 children not resident in France during the 15 days before the onset of the disease were removed from the analysis.

The average incidence of HUS was 0·7/100000 children < 15 years during the 3-year period (Table 1). A seasonal variation was observed, with most cases (59%) occurring in the summer between June and September (Fig. 1).

The children ranged in age from 1 month to 15 years with a median age of 24 months. Two hundred (81%) were less than 5 years of age. The highest incidence rate was observed in children up to 1 year of age (3·2/10^5 children per year) with 89 cases. The incidence for the group aged less than 5 years was 1·8/10^5 children per year, more than 8 times the incidence in those aged between 5 and 14 years (0·2/10^5 children per year) (Table 2). The number of cases among males and females was similar (140:146).

Table 3. E. coli *serogroups detected by serology associated with HUS* (*France, April 1995–March 1996*)

E. coli serogroup	Number	%
O157	68	57·6
O157 and O103	11	9·4
O103	5	4·3
O9	1	0·8
O105	1	0·8
O103 and O145	1	0·8
Negative for the 26 serogroups tested	31	26·3
Total	118	100

Clinical features

Information on the clinical features was available for 286 patients during the two study periods. Two hundred and fifty-eight (90%) of the 286 children had a prodromal diarrhoea, which was described as bloody for 149 (58%). Half of the patients with diarrhoea had been hospitalized at the time of diarrhoea. The mean time from onset of diarrhoea to diagnosis of HUS was 5·5 days (range 0–24 days). Four (1·4%) of the 286 children died during the acute phase of HUS. In all cases, death was attributed to CNS involvement.

Of the 286 patients, 130 were identified during the prospective study and for whom extra clinical data was available. Antibiotics were used before the diagnosis of HUS for 44 (37%) of the 119 patients with diarrhoea during the prospective study, more frequently for those with bloody diarrhoea (46 *vs.* 22%, $P = 0{\cdot}01$). Anti-motility agents were prescribed for 33 (28%) patients, more frequently for those who had bloody diarrhoea before the diagnosis of HUS (35 *vs.* 16%, $P = 0{\cdot}03$).

Dialysis was required for 53% (69/130) of patients. Most cases (112/130) received blood transfusion. Acute complications occurred in 25 (19%) patients, mostly central nervous system (CNS) and/or intestinal involvement.

Exposure to risk factors

Most of the cases were sporadic. Seven clusters were identified and investigated: one case in a village where

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Table 4. *Evidence of STEC infection in HUS cases* (*France, April 1995–March 1996*)

Evidence of STEC infection	Total of cases	With diarrhoea	Without diarrhoea
	Number (%)	Number (%)	Number (%)
STEC isolation	32/110 (29)	30/102 (30)	2/8 (25)
Stx genes	64/110 (58)	61/102 (60)	3/8 (38)
Antibodies to LPS	87/118 (74)	85/110 (77)	2/8 (25)
Antibodies to LPS O157	79/118 (67)	78/110 (71)	1/8 (12)
Any	105/122 (86)	101/113 (89)	4/9 (44)

4 other children had had HUS during the 2 previous years [2] were linked with the consumption of raw milk products; 2 brothers with HUS during the same week; 2 cousins with HUS within 2 days; 3 children in the same city with HUS during the same month and 3 occurrences of a HUS case associated with diarrhoea in family members. No outbreak of diarrhoea was detected around these clusters. In all these clusters, no evidence for a common food or environmental source of infection was identified.

Microbiology

Faecal specimens were available for 110/130 (85%) patients in the prospective study, and were collected within a mean of 7·5 days (range 0–24 days) after the onset of diarrhoea.

Stx genes were detected in stool samples of 64 (58%) cases, mainly stx_2 gene (59/64). *eae* gene was detected in 37 (63%) and *e-hlyA* gene in 30 (51%) of the 59 cases examined. The results of the PCR techniques were identical for 84 (82%) of the 103 samples analysed by the two laboratories concerning the detection of *stx* genes and for 77 (75%) concerning the type of Shiga toxins (Stx1, Stx2, Stx1 + Stx2). PCR positive and PCR negative patients did not differ in prodromal diarrhoea, bloody diarrhoea and delay between onset of diarrhoea and stool collection.

An *E. coli* strain positive for Shiga toxin was isolated from the stools of 32 (29%) of the 110 patients: 16 (50%) isolates were identified as O157:H7 serotype, and 3 as O103, O126 and O26. The 13 remaining isolates were untypable.

Serology

At least two serum samples were obtained from 91% (118/130) of the patients. Antibodies to LPS were detected in the sera of 87 (74%). *E. coli* serogroup O157 was found in 79 (67%) cases and *E. coli* serogroup O103 in 17 (14%) (Table 3).

Antibodies to LPS developed in 77% (85/110) of the patients with prodromal diarrhoea and in 25% (2/8) of those without diarrhoea ($P < 0{\cdot}005$).

The kinetics of anti-LPS antibodies were studied in the 57 patients with a positive serodiagnosis and for whom 3 sera were available. IgM antibodies were detected in 90% of the patients at D0, 93% at D15 then declined to 46% at D40. A rapid decrease of the proportion of patients with IgA antibody was observed from 87% at D0 to 32% at D15 and 2% at D40. The proportion of patients with IgG antibody remained stable (62% at D0 and D15, 55% at D40).

Evidence of STEC infection

Stool samples and/or sera were available for 122 (94%) of the 130 HUS cases from the prospective study. The combined microbiological and serological procedures provided evidence of STEC infection in 105 (86%) of 122 HUS cases (Table 4).

Results of serology and PCR were compatible in 57 (54%) out of the 106 cases with both serology and PCR results available. For 33 (31%) cases, the diagnosis of STEC infection relied only on serological findings and for 16 (15%) cases, only the stool PCR was positive.

Cases with STEC infection did not differ from other cases regarding demographic characteristics, clinical features and exposure to risk factors.

DISCUSSION

In this national study, the estimate of the annual incidence, mean age of cases, and the summer seasonality observed are comparable with reports

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from other European countries [8, 9]. The apparent increase in incidence between 1993 and 1994 must be interpreted with caution as this may be linked to the retrospective collection of information and the difficulty in reviewing the medical records. Following the study, a surveillance system of HUS was implemented. The incidence rate for 1996 was $0{\cdot}8/10^5$ children (99 cases), which indicates a stable incidence for the period 1994–1996 (Réseau National de Santé Publique, unpublished data).

The incidence rates were calculated only from cases notified by the paediatric nephrologists. Because of the clinical severity of HUS, most cases are probably referred to specialized units. In the prospective phase of the study, all French paediatric departments had been asked to participate. They notified only 17 cases. Including these cases, the HUS incidence rate was 1·1 cases per 100000.

The clinical features were comparable with those reported in other studies [10, 11]. In spite of progress in therapy, HUS is still a severe disease. Extrarenal complications, observed in 19% of the children, are known to have a poor prognosis both in the acute phase and in the long run [12].

Stx genes were detected in the stools of 58% of patients. Several factors could account for this low proportion such as transportation and freezing of specimens, and the interval between the onset of diarrhoea and stool collection [13]. Results of the PCR procedures concerning the type of Shiga toxins were similar for 75% of the samples tested. The discordant results provided by the two laboratories could be due to each using a different PCR prodecure. As all the children included were HUS cases, all the positive results have been taken into account. A comparison of the different PCR procedures was a secondary objective of this study and the results have been published elsewhere [4].

Antibodies to LPS were present in the sera of 74% of patients and were the sole evidence of STEC infection in 31% of cases. This confirms that the demonstration of a specific immune response may help to improve diagnosis of STEC infection in patients with HUS whose stool cultures are negative for STEC, as emphasized by other investigators [14]. The use of LPS from many different *E. coli* serogroups provides evidence that in France, as in other countries, O157 is the most prevalent serogroup involved in the pathogenesis of HUS [15, 16]. However, HUS can be associated with non-O157 STEC as has been described in several outbreaks and recent studies [17–19].

In 16 (15%) patients, the PCR results were positive, but the serodiagnosis was negative. One explanation could be that some of the STEC involved did not belong to one of the 26 serogroups tested.

Three different diagnostic criteria for STEC infection have been used in our study and evidence of infection by STEC has been found for the majority (86%) of the HUS cases, with a high proportion due to O157 serogroup. This result agrees with reports from other countries and confirms that in France STEC and *E. coli* O157 play an important role in the aetiology of this disease [15, 20].

In conclusion, this study has shown that the HUS incidence rate in France has been stable for 3 years and is around $1/10^5$ amongst children under 15 years of age. Most cases of HUS occurring in France were associated with STEC infection with a high prevalence of the O157 serogroup. However, some HUS cases were also associated with infection by non-O157 STEC. Following this study, we have implemented an active surveillance system based on reports from paediatric nephrologists to monitor the incidence of STEC infection and detect outbreaks. Monitoring of the incidence of HUS in children is a reliable indicator of trends in STEC infection, however, for the early detection of outbreaks the culture of stools from cases of diarrhoea is recommended [21, 22]. Further studies will help to establish the importance of this pathogen in the aetiology of diarrhoea in France and to establish further recommendations for the surveillance and the prevention of STEC infections.

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