Hemolytic uremic syndrome in Belgium: incidence and association with verocytotoxin-producing *Escherichia coli* infection

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Objective: To evaluate the incidence of hemolytic uremic syndrome (HUS) in Belgium and to determine the role of verocytotoxin-producing *Escherichia coli* O157:H7 and other serotypes (non-O157 VTEC).

Methods: Twenty-two centers, including the seven university hospitals, registered prospectively all cases of HUS; they collected clinical samples for isolation of VTEC strains and serum for detection of specific O-lipopolysaccharide antibodies.

Results: Forty-seven cases of HUS (including five incomplete cases) were recorded. Three cases were seen in non-residents. The incidence of complete HUS in Belgian residents was 4.3 cases/100 000 in children <5 years old, 1.8 cases/100 000 when all children <15 years were considered, and 0.42/100 000 when patients of all ages were taken into account. By combining bacteriologic and serologic results, evidence of VTEC infection was obtained in 64% of the patients, mainly but not exclusively in children with prodromal diarrhea. The 13 VTEC isolates belonged to serotypes O157:H7 (nine isolates), O26:H11, O121:H−, O145:H− and O172:H− (one each) and all produced VT2 (+VT2vh-a in three O157 strains) and were positive for the *eaeA* gene.

Conclusions: The incidence rate found in this study and the high mortality and morbidity linked with this syndrome warrant further registration of pediatric and post-diarrheic adult HUS cases and also examination of stools for both O157 and non-O157 VTEC strains. For effective prevention of this disease, further study of the serotypes and accessory virulence factors associated with HUS is needed.

Key words: Hemolytic uremic syndrome, verocytotoxins, Shiga toxins, verocytotoxin-producing *E. coli* (VTEC), Shiga toxin-producing *E. coli* (STEC), HUS incidence, HUS surveillance

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INTRODUCTION

The hemolytic uremic syndrome (HUS) is the main cause of acute renal failure in childhood [1]. It not only causes acute morbidity and mortality but can also be responsible for long-term sequelae. In a recent report with a follow-up of 10 years, in addition to 6% of children who died in the acute phase and 4% who went into end-stage renal failure, as many as 9% of survivors presented severe sequelae and 26% mild defects [2]. Cases are generally divided into typical (preceded by diarrhea) and atypical (without diarrhea). The latter presentation is rare in children and it is generally considered to have a poor prognosis [1]. Thrombotic thrombocytopenic purpura (TTP), considered to be the opposite end of the same spectrum, is characterized by the additional presence of fever and fluctuating central nervous system abnormalities [1]. Karmali et al have shown that post-diarrheic HUS cases are associated with strains of *Escherichia coli* that produce cytotoxins called verocytotoxins (VTs) or Shiga toxins [3,4]. These toxins are also produced by *Shigella dysenteriae* type 1, and this species is responsible for most cases of HUS in developing countries [5]. More than 100 serotypes of *E. coli* can produce VTs [6]. The most frequently isolated VT-producing *E. coli* (VTEC) serotype, O157:H7, and strains which manifest the same clinical, epidemiologic and pathogenetic features, e.g. serotypes O26:H11 and O111:H−, are also known as enterohemorrhagic *E. coli* (EHEC).

The epidemiology of HUS has mainly been studied in North America and the UK, where its incidence seems to be increasing. In these countries, mainly the O157:H7 VTEC serotype is involved in HUS [7]. In continental Europe, the incidence of HUS is lower and contrasting data have been published concerning the respective roles of O157:H7 and other VTEC serotypes [8]. The objective of the present study was to evaluate the incidence of HUS in Belgium and to determine the role of O157 VTEC serotypes (including O157:H7 and its non-motile variant O157:H−) and of other serotypes (non-O157 VTEC).

MATERIALS AND METHODS

Participating centers

The members of the Belgian Society for Infectious Diseases and Clinical Microbiology as well as the pediatric nephrology centers of the Belgian university hospitals were asked to join a multicenter study group and to register prospectively all cases of HUS diagnosed in their center during the period from 1 January to 31 December 1996. Twenty-two centers, formally registered as participants, including the seven Belgian university hospitals, were contacted several times to make sure that all cases were effectively reported.

Case definition

HUS was defined as an acute illness in a previously healthy patient characterized by hemolytic anemia with evidence of erythrocyte fragmentation on peripheral blood smear, thrombocytopenia defined by a platelet count of less than 160 000/μL, and renal failure defined by a serum creatinine level higher than 1.0 mg/dL for children less than 15 years old and higher than 2.0 mg/dL for adults. Cases not fulfilling all these criteria could be recorded as incomplete cases.

Case reports

For each case, the participants were asked to complete a questionnaire including demographic, clinical and laboratory data. A second questionnaire was sent for assessment of short-term follow-up after 3 months or more if needed.

Collection of samples for the diagnosis of VTEC infection

Participants were asked to send clinical samples to the VTEC reference laboratory for isolation of VTEC strains, and serum for detecting specific O-lipopolysaccharide antibodies. For VTEC isolation, a fecal specimen, a rectal swab or a primary enteric agar plate had to be sent as early as possible after diagnosis to the reference laboratory. Serum samples had to be collected in the acute phase and, when possible, a few weeks later.

Isolation of VTEC from feces and characterization of *E. coli* isolates

VTEC strains were searched for with a PCR technique previously described [9]. Briefly, a suspension of a colony sweep taken from the primary MacConkey agar plate (or other enteric agar) was used both without further treatment and after immunomagnetic separation (Dynabeads anti-*E. coli* O157, Dynal, Oslo, Norway) in a PCR reaction using a consensus primer pair amplifying VT1, VT2 and its variants [10]. A sample was considered positive only if at least two repeats of the PCR reaction starting from the same plate were positive. For each PCR-positive plate, 10–100 colonies were tested separately in order to isolate the VTEC strain in pure culture for further characterization. After biochemical identification, the isolates were agglutinated with O157 antiserum and tested on the H7 antiserum–sorbitol fermentation medium [11]. Further serotyping of non-agglutinating strains and phage typing of O157 strains were performed by Dr B. Rowe, PHLS, London, except for one strain serotyped by Dr F. Scheutz, Statens Seruminstitut, Copenhagen, Denmark. Production of

VT was confirmed by cytotoxicity testing on Vero cells [12]; the genotype of the B subunit was determined with specific PCR primers [13,14]. Accessory virulence factors were detected as previously described [9] by using phenotypic tests for *E. coli* hemolysins [15] and PCR for the *eaeA* gene [16] and for sequences of the 60-MDa virulence plasmid [17].

Detection of specific lipopolysaccharide antibodies

An indirect hemagglutination assay was used to detect antibodies against the lipopolysaccharidic antigens (LPS) of 11 *E. coli* O serogroups—O157, O2, O26, O91, O103, O111, O113, O128, O121, O145 and O172—and of *Shigella dysenteriae* type 1. These *E. coli* serotypes were selected because they were identified frequently in other HUS studies [7,18], in HUS cases in Belgium [12] (this study) or in other human VTEC infections in Belgium [9]. The LPS was prepared as described by Siddons and Chapman [19], using strains isolated in our laboratory [9,12] (this study). The resulting antigen preparation was stored at −20°C and used to sensitize lyophilized red blood cells as described by Bitzan and Karch [20]. Unsensitized red blood cells were used as control with each investigated patient's serum. The indirect hemagglutination was performed as described by Bitzan and Karch, but we started with a serum dilution of 1:8 or more if the available volume of serum was limited. On the basis of the data of these authors and preliminary unpublished observations from our laboratory showing that control patients had only exceptionally reverse titers of 256 or higher, we considered a reaction as positive if a titer of 256 or more was observed.

RESULTS

During the 1-year study period, 47 cases of HUS were recorded in 14 of the 22 participating centers. Five pediatric cases were incomplete because they did not fulfill one of the case definition criteria, i.e. the elevated creatininemia. Three patients were non-residents transferred to Belgium for therapy. Of the 44 resident cases, two were traveling in the Canary Islands when they fell ill. Thirty-nine cases of complete HUS were recorded in patients residing in Belgium. The incidence of complete HUS in Belgian residents computed on the basis of population data from the National Institute of Statistics was 4.3 cases/100 000 in children <5 years old, 1.8 cases/100 000 when all children <15 years old were considered, and 0.42/100 000 when patients of all ages were taken into account. To our knowledge, all these cases except two siblings were unrelated. They were distributed among all Belgian provinces. No clustering in time was observed. Demographic characteristics of all recorded cases are presented in Table 1. In 38 of the 47 patients (34 of the 38 children, and four of the nine adults), a prodromal episode of diarrhea was reported. Diarrhea was bloody in 20 children but in none of the adult cases. In two children without diarrhea, a prodromal phase of nausea and vomiting was reported. One 4-year-old mentally handicapped boy died during transportation to the hospital, and the diagnosis was suspected because his sister developed incomplete HUS 5 days later and had O157:H7 VTEC isolated from her stools. Necropsy findings in the boy—signs of gastro-enteritis with reactional mesenteric lymph nodes and

Table 1 Demographic characteristics of the HUS cases

	No. of patients with prodromal diarrhea	No. of patients without prodromal diarrhea	All patients
Sex			
Female	18[a] (1)[c]	4	22[a] (1)
Male	20 (4)	5[b]	25[b] (4)
Age (years)			
< 1	5[b] (1)	1	6[b] (1)
1–4	23[b] (4)	3	26[b] (4)
5–14	6	0	6
15–24	0	0	0
25–34	1	1	2
35–44	2	1	3
45–54	0	1	1
55–64	0	1[b]	1[b]
>65	1	1	2
Total	38 (5)	9	47 (5)

[a] Includes two non-resident cases.

[b] Includes one non-resident case.

[c] The numbers in parentheses represent the number of incomplete HUS cases.

presence of microthrombi in the kidneys and lungs—were compatible with the diagnosis. The biochemical data on 44 of the 46 other patients (complete data not being available in two cases) were characteristic of HUS: all had a hemoglobin level of less than 10 mg/dL (median of the lowest values 6.8 mg/dL, range: 3.4–9.6 mg/dL); schizocytes were present in all but four, where they were not looked for; all had thrombocyte counts lower than 160 000/μL (median of the lowest platelet counts 32 250/μL, range <1000–118 000/μL); and all but the five children with incomplete HUS had elevated creatininemia (median of the highest value in children 3.7 mg/dL, range 0.6–12.0 mg/dL; median of the highest value in adults: 6.1 mg/dL, range 3.5–10.9 mg/dL).

Follow-up data after 3 months were available in 41 patients. The following complications were recorded. Five patients (four children and one adult) had severe colitis, three (one child and two adults) pancreatitis, two (one child and one adult) septic shock and two (both adults) pulmonary hemorrhage. In addition to the child who died during transfer to the hospital, three adults died, one in septic shock and two from pulmonary hemorrhage. The hospital stay in surviving patients ranged from 7 to 56 days in children (median 16 days) and from 11 to 179 days in adults (median 30 days). Among the 34 surviving patients with available follow-up data at 3 months, seven (six children and one adult) still showed hypertension, five (four children and one adult) renal insufficiency, seven (all children) proteinuria and one (child) hematuria.

Stool samples were available in 33 cases. Fourteen (42%) yielded a positive PCR, in one case only after immunomagnetic separation. The serotypes and virulence factors of the 13 VTEC strains that could be isolated, nine O157:H7 and four other serotypes, are presented in Table 2. In the last PCR-positive sample, no VTEC could be detected among 100 separately tested colonies. In addition, urine and blood *E. coli* isolates from one adult patient from whom no stools were available were PCR negative. In 13 of the 34 patients from whom serum samples were available (more than one sample in only six patients), the hemagglutination reaction was positive for at least one of the O antigens, with a reverse titer higher than or equal to 256. Eleven patients were positive for O157 (median reverse titer 2096, range 256–16 384), one for O111 (titer: 8192) and one for both O26 and O157 (titers of 1024 against both antigens). In addition, the results were not interpretable in two patients presenting non-specific agglutination of unsensitized red blood cells.

When the results of the PCR and VTEC isolation from stools were combined with serology, 23 of 36 patients from whom either stools, serum or both were available had evidence of VTEC infection, including the two children without diarrhea but with vomiting and anorexia. When only children or patients with diarrhea were taken into account, 22 of 30 and 21 of 29, respectively, showed evidence of VTEC infection. Only one adult patient, a 69-year-old woman with prodromal diarrhea, had a positive VTEC result (anti-O157 LPS titer of 4096).

DISCUSSION

Data on the incidence of HUS are only available from countries of Europe and North America as well as from Argentina. Table 3 shows a comparison of the published HUS incidence rates in different countries [21–35]. Even after exclusion of Argentina, which has a much higher incidence than any other country [35], the range is large: 1.08–5.8 cases/100 000 in children less than 5 years old and 0.2–2.0 in children less than 15 years old. No data are available on the incidence in adults [7]. Variations in data-collection methods may account for part of this variation and could, for instance, explain the important discrepancy between the data from four French departments in 1982–93 [23] and those from the whole of France in 1993–95 [22].

Table 2 Serotypes and virulence factors of the 13 VTEC isolates from HUS cases

Serotype	Number	VT2	VT2 + VT2vh-a	*eaeA* gene	Entero-hemolysin	α-Hemolysin	No hemolysin	Virulence plasmid
O157:H7 [a]	9	6	3	9	9	0	0	9
O26:H11	1	1	0	1	1	0	0	1
O121:H−	1	1	0	1	1	0	0	1
O145:H−	1	1	0	1	0	1	0	1
O172:H−	1	1	0	1	0	0	1	1
Total	13	10	3	13	11	1	1	13

[a] Belonging to the following phage types (PT): PT 2 (two strains), PT 4 (three strains), PT 49 (three strains) or RDNC (reaction does not conform to the phage types defined until now; one strain).

Table 3 Comparison of the annual incidence of HUS in different countries

Country	Incidence per 100 000 children <5 years old	Incidence per 100 000 children <15 years old	Year(s) studied	Reference
Europe				
Austria	NR	0.37	1995	[21]
Belgium	4.3	1.8	1996	This study
France	1.78	0.72	1993–95	[22]
France (four departments)	4.61	NR	1982–93	[23]
Germany (northern part)	2.7	1.0	1986–91	[24]
Italy	NR	0.2	1988–92	[25]
The Netherlands	2.0	NR	?	[26]
UK	NR	0.79	1985–88	[27]
North America				
Canada	3.11	1.44[a]	1986–88	[28]
Canada (Alberta)	4.5	NR	1987–91	[29]
USA (King County, Washington)	NR	0.69	1971–75	[30]
USA (King County, Washington)	NR	1.77	1976–80	[30]
USA (King County, Washington)	NR	1.74	1981–86	[30]
USA (Maryland and Washington DC)	1.08	0.26[b]	1979–83	[33]
USA (Minnesota)	1.6	0.5[a]	1979	[32]
USA (Minnesota)	5.8	2.0[a]	1988	[32]
USA (Oregon)	2.65	0.97[c]	1979–82	[31]
USA (Utah)	NR	1.42	1971–90	[34]
South America				
Argentina (Buenos Aires and suburbs)	21.7	NR	1989	[35]

[a]From 0.97 (Ontario) to 2.78 (Alberta); [b]children <21 years old; [c]children <18 years old.
NR, not reported.

The incidence rates that we determined in this Belgian study are closer to the North American rates, which tend to be higher than those observed in European countries. This could be due in part to a more complete recording of cases thanks to the active follow-up in the present study: several reminders were sent to all participants and, in addition, centers reporting no or only a few cases were telephoned. However, the present incidence could be still higher in North America and the UK, since most data from these countries date from the 1970s and the 1980s, while the numbers of reported sporadic VTEC infection cases and of outbreaks are increasing [7,29,36,37].

Evidence of VTEC infection could be demonstrated in 23 of 36 patients from whom either stools or serum or both were available. When only children or patients with diarrhea were considered, the proportions increased to 22 of 30 and 21 of 29 respectively. These figures are rather low, as compared, for instance, with French data, where 86% of children with HUS had a laboratory diagnosis of VTEC infection [22]. This does not mean, however, that none of the other patients had VTEC infection; it is known that, at the time of admission with HUS diagnosis, VTEC is no longer detectable in many patients [38], especially if antibiotics have already been given before stool collection, as in more than half of our patients for whom this information was available (data not shown). Moreover, the serologic investigations are limited in two ways: we investigated a restricted number of serotypes, and in many patients no late serum sample was available.

As already reported by other authors, it is worthwhile looking for VTEC infection even in cases where VTEC is not expected, as in patients without diarrhea [25] and in adults [39,40]. For instance, in our study, two children without diarrhea and a 69–year-old woman had VTEC infection. In addition, all urinary tract *E. coli* isolates from HUS patients should be tested for toxin production since HUS has recently been associated with cases of urinary tract infection [41,42].

In a screening for VTEC in unselected stool samples submitted for culture in the Academisch Ziekenhuis Vrije Universiteit Brussel, only one-fifth of all VTEC isolates belonged to serotype O157:H7 or its non-motile variant O157:H− [9]. However, in this HUS study, when the patient with a positive PCR but with no VTEC isolate was excluded, and the patient with double positive serologic tests was counted twice,

O157 infection was diagnosed in 17 of the 22 patients with laboratory-confirmed VTEC infection and non-O157 infection in only six. This predominance of serogroup O157 has also been observed in other European countries [8,22,24,43,44]. In France, although previous studies demonstrated a leading role for non-O157 VTEC in HUS [45,46], recent data showed O157 VTEC to be associated with 91% of HUS cases [22].

All 13 VTEC strains isolated here (including the four non-O157 isolates) produced the VT2-type toxin, combined with the VT2vh-a variant toxin in three O157:H7 strains, possessed the *eaeA* gene and the large-sized virulence plasmid, and produced enterohemolysin (except for two strains that were α- or non-hemolytic). VT2-producing non-O157 strains that also possess other virulence factors were only exceptionally isolated from unselected stool specimens: only three of 55 VT2 or VT1+VT2-positive strains were *eaeA* positive [9]. By contrast, most *eaeA*-positive strains produced VT1 and were more frequent in patients with diarrhea than in patients without diarrhea. The simultaneous presence of VT2 and the *eaeA* gene might explain the high pathogenicity of most O157 strains [47] and of many non-O157 VTEC strains isolated in HUS cases [46]. Of the four non-O157 serotypes, one of them, O26:H11, has already repeatedly been isolated from HUS [3] and is considered to be enterohemorrhagic. However, while VT2-producing O26:H11 strains have rarely been described, several HUS isolates presented this characteristic [46,48]. The three other serogroups were already identified in HUS cases, although rarely [3,12]. O172 is a recently established serogroup [49] but we isolated it in 1988 in a baby with HUS and reported this under its temporary denomination, O"C70:86" [12].

In conclusion, the HUS incidence rate found in this study and the high morbidity and mortality linked to this syndrome warrant further registration of pediatric and post-diarrheic adult HUS cases and also examination of stools for both O157 and non-O157 VTEC strains. Methods are available that detect not only non-O157 VTEC strains but also atypical sorbitol-positive O157 strains as described in Germany [24] and other organisms producing VTs that have sometimes been involved in HUS, such as *Citrobacter freundii* [50] and *Enterobacter cloacae* [51]. Further study of the *E. coli* serotypes and accessory virulence factors associated with HUS is needed to define better which VTEC isolates can cause this syndrome. This will contribute to a better understanding of the sources of infection, which seem to be different in continental Europe and in North America and the UK [8]. Implementation of efficacious quality control programs by food microbiologists will only be possible if these data are available.

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References

1. Proesmans W. Typical and atypical hemolytic uremic syndrome. Kidney Blood Press Res 1996; 19: 205–8.
2. Kelles A, Van Dyck M, Proesmans W. Childhood haemolytic uraemic syndrome: long-term outcome and prognostic features. Eur J Pediatr 1994; 153: 38–42.
3. Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet 1983; 1: 619–20.
4. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J Infect Dis 1985; 151: 775–82.
5. Srivastava RN, Moudgil A, Bagga A, Vasudev AS. Hemolytic uremic syndrome in children in northern India. Pediatr Nephrol 1991; 5: 284–8.
6. Lior H. Classification of *Escherichia coli*. In Gyles CL, ed. *Escherichia coli* in domestic animals and humans. Oxon, UK: CAB International; 1994: 31–72.
7. Griffin PM. *Escherichia coli* 0157:H7 and other enterohemorrhagic *Escherichia coli*. In Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL, eds. Infections of the gastrointestinal tract. New York: Raven Press, Ltd, 1995: 739–61.
8. Caprioli A, Tozzi AE. Epidemiology of Shiga toxin-producing *Escherichia coli* infections in continental Europe. In Kaper, JB, O'Brien A, eds. *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. Washington, DC: American Society for Microbiology, 1998: 38–48.
9. Piérard D, Stevens D, Moriau L, Lior H, Lauwers S. Isolation and virulence factors of verocytotoxin-producing *Escherichia coli* in human stool samples. Clin Microbiol Infect 1997; 3: 531–40.
10. Karch H, Meyer T. Single primer pair for amplifying segments of distinct Shiga-like toxin genes by polymerase chain reaction. J Clin Microbiol 1989; 27: 2751–7.
11. Farmer JJ, III, Davis BR. H7 antiserum-sorbitol fermentation medium: a single tube screening medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J Clin Microbiol 1985; 22: 620–5.
12. Piérard D, Van Etterijck R, Breynaert J, Moriau L, Lauwers S. Results of screening for verocytotoxin-producing *Escherichia coli* in faeces in Belgium. Eur J Clin Microbiol Infect Dis 1990; 9: 198–201.
13. Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J Clin Microbiol 1990; 28: 540–5.
14. Tyler SD, Johnson WM, Lior H, Wang G, Rozee KR. Identification of verotoxin type 2 variant B subunit genes in *Escherichia coli* by the polymerase chain reaction and restriction fragment length polymorphism analysis. J Clin Microbiol 1991; 29: 1339–43.
15. Beutin L, Montenegro MA, Orskov I, et al. Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. J Clin Microbiol 1989; 27: 2559–64.

16. Gannon VP, Rashed M, King RK, Thomas EJ. Detection and characterization of the *eae* gene of Shiga-like toxin-producing *Escherichia coli* using polymerase chain reaction. J Clin Microbiol 1993; 31: 1268–74.
17. Fratamico PM, Sackitey SK, Wiedmann M, Deng MY. Detection of *Escherichia coli* O157:H7 by multiplex PCR. J Clin Microbiol 1995; 33: 2188–91.
18. Karmali MA. Infection by verocytotoxin-producing *Escherichia coli*. Clin Microbiol Rev 1989; 2: 15–38.
19. Siddons CA, Chapman PA. Detection of serum and faecal antibodies in haemorrhagic colitis caused by *Escherichia coli* O157. J Med Microbiol 1993; 39: 408–15.
20. Bitzan M, Karch H. Indirect hemagglutination assay for diagnosis of *Escherichia coli* O157 infection in patients with hemolytic-uremic syndrome. J Clin Microbiol 1992; 30: 1174–8.
21. Allerberger F, Dierich MP. Enterohemorrhagic *Escherichia coli* in Austria. [abstract V37/I] In: Abstract book of the Third International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* Infections, Baltimore, Maryland, USA. Melville, NY: Lois Joy Galler Foundation for Hemolytic Uremic Syndrome Inc., 1997: 4.
22. Decludt B, Bouvet P, Mariani P, et al. Hemolytic uremic syndrome and verotoxin-producing *Escherichia coli* in France. [abstract V184/I]. In: Abstract book of the Third International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* Infections, Baltimore, Maryland, USA. Melville, NY: Lois Joy Galler Foundation for Hemolytic Uremic Syndrome, Inc., 1997: 21.
23. Cloarec S, Deschênes G, Besson-Leaud M, et al. Epidemiologie du syndrome hemolytique et urémique aigu postdiarréique de l'enfant dans quatre départements francais. Arch Pediatr 1996; 3: 1209–14.
24. Bitzan M, Ludwig K, Klemt M, Konig H, Buren J, Muller Wiefel DE. The role of *Escherichia coli* O 157 infections in the classical (enteropathic) haemolytic uraemic syndrome: results of a Central European, multicenter study. Epidemiol Infect 1993; 110: 183–96.
25. Gianviti A, Rosmini F, Caprioli A, et al. Haemolytic-uraemic syndrome in childhood: surveillance and case-control studies in Italy. Pediatr Nephrol 1994; 8: 705–9.
26. Van de Kar NC, Heuvelink AE, de Boer E, Monnens LA. Infecties met verocytotoxine-producerende *Escherichia coli* en het hemolytisch-uremisch syndroom. Ned Tijdschr Geneeskd 1996; 140: 134–7.
27. Milford DV, Taylor CM, Guttridge B, Hall SM, Rowe B, Kleanthous H. Haemolytic uraemic syndromes in the British Isles 1985–8: association with verocytotoxin producing *Escherichia coli*. Part 1: Clinical and epidemiological aspects . Arch Dis Child 1990; 65: 716–21.
28. Rowe PC, Orrbine E, Wells GA, McLaine PN. Epidemiology of hemolytic-uremic syndrome in Canadian children from 1986 to 1988. J Pediatr 1991; 119: 218–24.
29. Waters JR, Sharp JC, Dev VJ. Infection caused by *Escherichia coli* O157:H7 in Alberta, Canada, and in Scotland: a five-year review, 1987–1991. Clin Infect Dis 1994; 19: 834–43.
30. Tarr PI, Neill MA, Allen J, Siccardi CJ, Watkins SL, Hickman RO. The increasing incidence of the hemolytic-uremic syndrome in King County, Washington: lack of evidence for ascertainment bias. Am J Epidemiol 1989; 129: 582–6.
31. Rogers MF, Rutherford GW, Alexander SR, et al. A population-based study of hemolytic-uremic syndrome in Oregon, 1979–1982. Am J Epidemiol 1986; 123: 137–42.
32. Martin DL, MacDonald KL, White KE, Soler JT, Osterholm MT. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnestoa. N Engl J Med 1990; 323: 1161–7.
33. Kinney JS, Gross TP, Porter CC, Rogers MF, Schonberger LB, Hurwitz ES. Hemolytic-uremic syndrome: a population-based study in Washington, DC and Baltimore, Maryland. Am J Public Health 1988; 78: 64–5.
34. Siegler RL, Pavia AT, Christofferson RD, Milligan MK. A 20-year population-based study of postdiarrheal hemolytic uremic syndrome in Utah. Pediatrics 1994; 94: 35–40.
35. Lopez EL, Diaz M, Grinstein S, et al. Hemolytic uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. J Infect Dis 1989; 160: 469–75.
36. Armstrong GL, Hollingsworth J, Morris JG, Jr. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiol Rev 1996; 18: 29–51.
37. Wall PG, McDonnell RJ, Adak GK, Cheasty T, Smith HR, Rowe B. General outbreaks of vero cytotoxin producing *Escherichia coli* O157 in England and Wales from 1992 to 1994. Commun Dis Rep CDR Rev 1996; 6: R26–33.
38. Tarr PI, Neill MA, Clausen CR, Watkins SL, Christie DL, Hickman RO. *Escherichia coli* O157:H7 and the hemolytic uremic syndrome: importance of early cultures in establishing the etiology. J Infect Dis 1990; 162: 553–6.
39. Krishnan C, Fitzgerald VA, Dakin SJ, Behme RJ. Laboratory investigation of outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7. J Clin Microbiol 1987; 25: 1043–7.
40. Cowden JM. Scottish outbreak of *Escherichia coli* O157, November–December 1996. Eurosurveillance 1997; 2: 1–2.
41. Tarr PI, Fouser LS, Stapleton AE, et al. Hemolytic-uremic syndrome in a six-year-old girl after a urinary tract infection with Shiga-toxin-producing *Escherichia coli* O103:H2. N Engl J Med 1996; 335: 635–8.
42. Reymond D, Bianchetti MG, Burnens A, Lior H. Haemolytic-uraemic syndrome. Lancet 1994; 343: 1042–3.
43. Caprioli A, Luzzi I, Rosmini F, et al. Hemolytic-uremic syndrome and Vero cytotoxin-producing *Escherichia coli* infection in Italy. J Infect Dis 1992; 166: 154–8.
44. Van de Kar NC, Roelofs HG, Muytjens HL, et al. Verocytotoxin-producing *Escherichia coli* infection in hemolytic uremic syndrome in part of western Europe. Eur J Pediatr 1996; 155: 592–5.
45. Mariani Kurkdjian P, Denamur E, Milon A, et al. Identification of a clone of *Escherichia coli* O103:H2 as a potential agent of hemolytic-uremic syndrome in France. J Clin Microbiol 1993; 31: 296–301.
46. Mariani Kurkdjian P, Cave H, Elion J, Loirat C, Bingen E. Direct detection of verotoxin genes in stool samples by polymerase chain reaction in hemolytic uremic syndrome patients in France. Clin Microbiol Infect 1997; 3: 117–19.
47. Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J Infect Dis 1989; 160: 994–8.
48. Ricotti GC, Buonomini MI, Merlitti A, Karch H, Luzzi I, Caprioli A. A fatal case of hemorrhagic colitis, thrombocytopenia, and renal failure associated with verocytotoxin-producing, non-O157 *Escherichia coli*. Clin Infect Dis 1994; 19: 815–16.
49. Orskov I, Wachsmuth IK, Taylor DN, et al. Two new *Escherichia coli* O groups: O172 from 'Shiga-like' toxin II-producing strains (EHEC) and O173 from enteroinvasive *E. coli* (EIEC). APMIS 1991; 99: 30–2.
50. Tschäpe H, Prager R, Streckel W, Fruth A, Tietze E, Bohme G. Verotoxinogenic *Citrobacter freundii* associated with severe gastroenteritis and cases of haemolytic uraemic syndrome in a nursery school: green butter as the infection source. Epidemiol Infect 1995; 114: 441–50.
51. Paton AW, Paton JC. *Enterobacter cloacae* producing a Shiga-like toxin II-related cytotoxin associated with a case of hemolytic-uremic syndrome. J Clin Microbiol 1996; 34: 463–5.