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A community outbreak of haemolytic-uraemic syndrome in children occurring in a large area of Northern Italy over a period of several months

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SUMMARY

From March to October 1993, 15 cases of haemolytic-uraemic syndrome (HUS) in children were detected in a large area of northern Italy, where only 8 cases had occurred in the previous 5 years. Analysis of stool and serum specimens obtained from 14 cases showed evidence of Verotoxin-producing *Escherichia coli* (VTEC) infection in 13. Serum antibodies to the *E. coli* O157 lipopolysaccharide (LPS) were found in 8 patients and to the O111 LPS in 2. An O86 VTEC was isolated from another patient. Fourteen children needed dialysis, and 1 died. No obvious epidemiologic link was observed among cases, most of whom lived in small townships. A case-control study did not show an association between HUS and food or exposure to cattle, but suggested an association with contact with chicken coops (OR = 6·5, 95% C.I. 1·2–34·9). However, VTEC were not isolated from stool samples obtained from the chicken coops involved. The risk factors for VTEC infection related to living in rural settlements, including the exposure to live poultry, should be considered in outbreak investigations.

INTRODUCTION

Verotoxin producing *Escherichia coli* (VTEC), particularly strains belonging to serogroup O157, are an emerging cause of diarrhoea and haemorrhagic colitis in western countries. According to Griffin and Tauxe [1], between 2–7% of persons infected with *E. coli* O157:H7 develop haemolytic uraemic syndrome (HUS), which is characterized by the association of renal failure, thrombocytopenia and haemolytic microangiopathic anaemia.

Outbreaks of VTEC infection have been described in recent years in North

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America and the UK, and have become an important public health problem [2–7]. The last major epidemic occurred in the west coast of USA at the beginning of 1993 [8]. It involved approximately 600 confirmed cases of infection with *E. coli* O157:H7 and received widespread coverage in the lay press.

In Italy, a nationwide surveillance system for HUS in paediatric patients was established in 1988. On average, 0·2 HUS incident cases per 100000 per year in the age range 0–15 years were reported between 1988 and 1992 (unpublished observations). Laboratory investigations showed that 75% of the HUS cases detected by this surveillance system were attributable to VTEC infection. The most common serogroups were O157 (51% of cases), followed by O26 (8%), and O111 (4%) [9].

Foods of bovine origin, such as raw beef, unpasteurized milk and yoghurt [1–4, 8, 10], and other contaminated foods and beverages [5, 6, 11] have been identified as vehicles of *E. coli* O157 infection in other countries. In Italy, a case-control study on sporadic cases of HUS demonstrated a significant association of the disease with the occurrence of diarrhoea among family contacts of cases some days before illness, but not with the consumption of any specific food (unpublished observations). In 1992, the routine surveillance system detected a community outbreak associated with *E. coli* O111 infection. A total of 9 HUS cases were identified in northern Italy, but the epidemiologic investigation failed to demonstrate any common source of infection [12].

Although the investigations conducted to date have not demonstrated food-borne transmission of VTEC infection, recent studies indicated that cattle and cattle products may represent an important reservoir of VTEC in Italy. Verotoxin producing *E. coli* serogroups previously associated with human disease have been isolated from healthy and sick calves [13], and 9% of 144 retail ground beef samples were found to have VT in enrichment culture supernatants [14].

During summer 1993 a higher number of HUS cases was notified to the surveillance system from an area in northern Italy including 3 contiguous regions out of the 20 in the country, namely Veneto, Emilia and Trentino. We describe here the features of this unusual outbreak, in which the only cases that could be identified were children with HUS.

METHODS

Case definition

The cases were considered in the time interval between March and October 1993 among residents in the contiguous Veneto, Emilia and Trentino regions. A case was defined as a patient with HUS. According to the clinical definition of the surveillance system protocol, diagnosis of HUS was based on the appearance of pallor or oliguria and by laboratory evidence of intravascular haemolysis, thrombocytopenia (platelet count $< 100 \times 10^9$/l), and evidence of renal involvement with at least 2 of the following: blood urea nitrogen $> 7{\cdot}1$ mmol/l, serum creatinine > 71 μmol/l, or abnormal urinary sediment test. Patients with diarrhoea or bloody diarrhoea, and laboratory evidence of VTEC infection observed in the same period as patients with HUS, were also considered as cases.

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Case finding

HUS cases were routinely notified to the surveillance system [9]. We searched for other cases among patients of any age admitted to the same hospital as the persons with HUS in the period March–October 1993 by discussion with hospital-based physicians and reviewing discharge records. Patients with one of the following diagnoses at discharge were investigated: non-specific gastroenteritis (ICD IX 003·9), colitis, enteritis and gastroenteritis of presumed viral origin (ICD IX 009·3), other non-infectious gastroenteritis and colitis (ICD IX 558), rectal and anal bleeding (ICD IX 569·3), abnormal stools (ICD IX 787·7), symptoms of the intestinal apparatus (ICD IX V100).

In September 1993 the National Paediatric Congress and other minor meetings were held in the outbreak area, and short or informal communications were dedicated to the description of the ongoing outbreak. The physicians participating in these meetings were invited to notify suspected cases of VTEC infection.

Case control study

The families of cases were asked to provide the names, addresses and telephone numbers of 2 non-related age-matched neighbours (± 6 months if less than 1 year of age; ± 1 year if older) in order to perform a case-control study on risk factors for the disease. Controls were eligible only if they had no gastrointestinal symptoms in the 3 months before the onset of symptoms in the case patient.

All cases and controls were interviewed using a similar standardized questionnaire administered in person or by telephone by two of the authors. The questionnaire was focused on dietary habits, exposure to farm animals, presence of household contacts with diarrhoea and travel in the 2 weeks before the onset of symptoms.

The data were entered in Epi-Info version 5 and the data analysis was performed by Mantel and Haenszel test for matched data with variable matching ratio [15].

Other investigations

Faecal specimens of all household contacts of the cases were requested for culture to identify possible carriers of VTEC. In addition, the butchers where the cases' families had bought beef in the 2 weeks before the onset of symptoms were identified. Then the list of their wholesale distributors in the month before the onset of symptoms was requested in order to identify possible common sources of contamination. Finally, in case of exposure of a case to farm animals in the 2 weeks before the onset of symptoms, animal faeces were collected, if possible, and cultured for VTEC.

Laboratory tests

Stool and serum specimens of cases were collected as soon as possible after diagnosis of HUS according to our surveillance protocol [9]. Sera were also obtained from suspected cases with both bloody and non-bloody diarrhoea, and stools from household contacts of HUS cases. The Vero cell assay was used to examine faeces for the presence of VTEC and free VT, and sera for VT-neutralizing

antibodies [9]. Verotoxins were identified by using rabbit VT-neutralizing antisera as previously described [9]. Sera were also examined for antibodies to the lipopolysaccharide (LPS) of *E. coli* O157 by ELISA [9, 16]. The same procedure was adapted to detect antibodies to the LPS of *E. coli* O26 and O111 [12]. The specificity of the ELISA test was confirmed by immunoblot as previously described [12].

The presence of VTEC in animal stools was assessed as previously described [13]. Briefly, freshly passed stools were seeded in trypticase soy broth and incubated overnight at 37 °C. The culture supernatants were tested by the Vero cell assay, and the presence of VT in supernatants causing a cytopathic effect was assessed by using rabbit neutralizing antisera to VT1 and VT2. For the VT-positive samples, the initial cultures in trypticase soy broth were inoculated onto MacConkey agar and colonies resembling *E. coli* were tested for VT production. VT-producing isolates were confirmed as *E. coli* by the API 20E system (Api; Biomerieux), and serogrouped by standard techniques using antisera to *E. coli* O157 and the enteropathogenic *E. coli* serogroups. Serotyping was confirmed and completed at the Escherichia International Reference Centre, Copenhagen, Denmark.

RESULTS

Case identification

From March to October 1993 15 patients with HUS were reported to the surveillance system from the study area mainly from paediatric nephrology centres. This represented a large increase over the 8 cases notified during the entire previous 5 years from the 3 regions involved (Fig. 1). The annual average incidence in the study area among residents aged 0–15 years in the period 1988–92 was 0·2 per 100000, compared with 1·6 per 100000 in 1993.

Eleven suspect cases with bloody or non-bloody diarrhoea were identified by the examination of hospital discharge lists and sera were collected from eight. None of them showed evidence of VTEC infection. No additional cases from other sources were notified, and no secondary cases were observed among household contacts of cases.

Laboratory findings

Stool and serum specimens were obtained from 14 of the 15 HUS cases on average 9 days (range 4–38) after the onset of prodromal symptoms. Evidence of VTEC infection was found in 13 of the 14 children examined. Antibodies to the O157 LPS were found in 8 patients, to O111 LPS in 2, and neutralizing antibodies to VT1 in 2 patients. A VT2-producing *E. coli* of serotype O86:H40 was isolated from one patient who was also positive for free faecal VT2. The patient had antibodies to the LPS of the infecting strain.

Geographical and temporal distribution

The geographical location of HUS cases residence is illustrated in Figure 2. The cases were distributed over a wide geographic area (17000 km^2) with a total population of 5000000 inhabitants. Twelve of the 15 lived in townships with less

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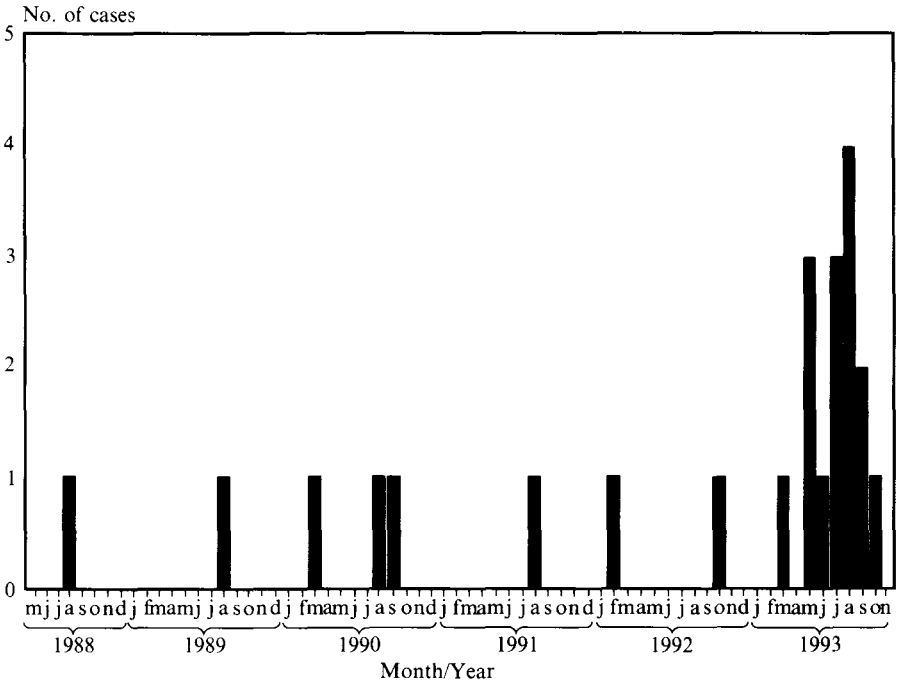

Fig. 1. Cases of haemolytic-uraemic syndrome in the study area by month of admission, May 1988 through November 1993.

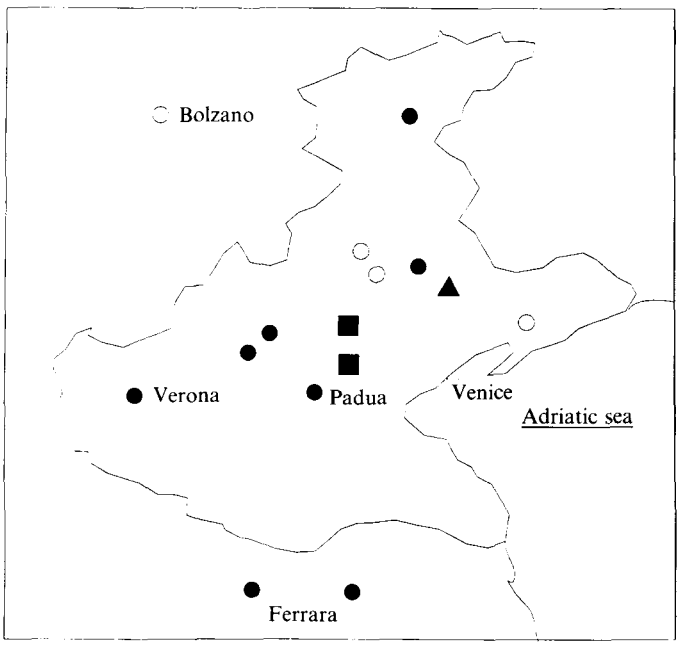

Fig. 2. Place of residence of haemolytic-uraemic syndrome by serogroup of the infecting VTEC strain. ● *E. coli* O157; ■ *E. coli* O111; ▲ *E. coli* O86; ○ undetermined.

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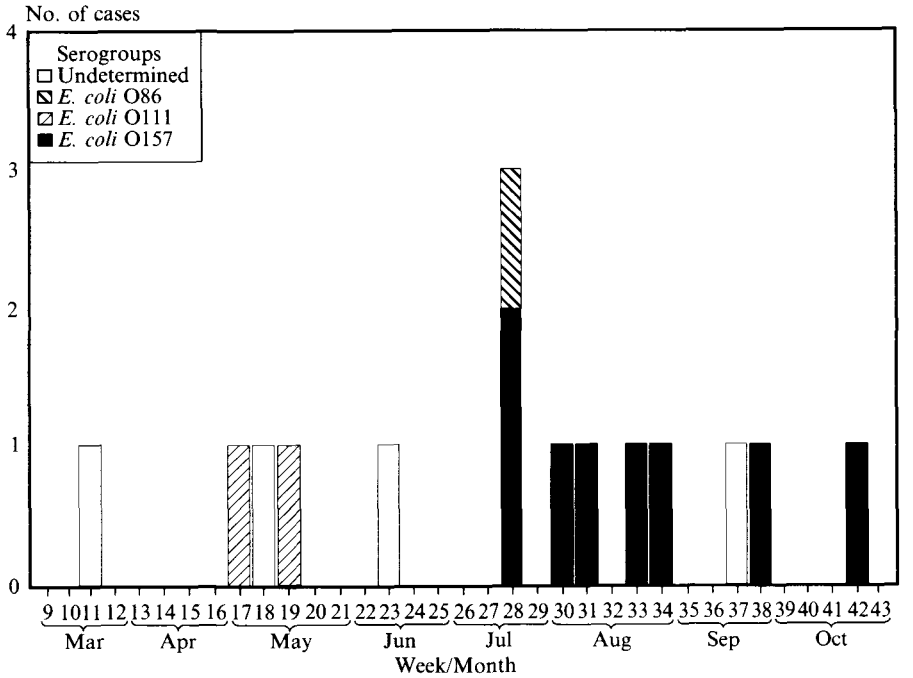

Fig. 3. Cases of haemolytic-uraemic syndrome by week of admission and serogroup of the infecting VTEC strain. March–October 1993.

than 20000 inhabitants. Three cases were seen over a 1 week period in July; the remaining cases were scattered over an 8-month period (Fig. 3).

The 2 cases with antibodies to *E. coli* O111 occurred in the first 2 weeks of May and lived about 5 km apart, while cases with evidence of infection with *E. coli* O157 occurred between August to October.

Clinical features

The median age of the 15 HUS cases was 3·5 years (range 4 months–10 years). The male-to-female ratio was 6:9. Prodromal bloody diarrhoea was observed in 9 patients, and non-bloody diarrhoea in 6 patients. The median duration of diarrhoea was 4 days (range 1–10) in patients with bloody diarrhoea, and 3 days (range 1–9) in those with non-bloody diarrhoea. Neurologic symptoms, primarily seizures and confused state, were observed in 5 patients. Twelve patients underwent dialysis. One patient had a permanent neurological damage with residual motor paresis and another one had died as of January 1994. Necroscopy of this last patient showed a circumferencial myocardial infarction with a microscopical picture of myocardial disarray and autolysis. She was 4-month-old at diagnosis, was the only one without evidence of VTEC infection, had repeated relapses of HUS and underwent dialysis for over 4 months.

Case-control study

Since no link was apparent among the cases or their households, a case-control study was conducted to identify common exposures. Two cases were able to report just 1 control, and therefore the case-control study included 15 cases and 28

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Table 1. *Results of the case-control study: odds ratios and 95% confidence limits*

Exposure	All cases ($n = 15$) OR	C.I. 95%	Cases with *E. coli* O157 infection only ($n = 8$) OR	C.I. 95%
Diarrhoea in households	Undetermined		Undetermined	
No. of households > 3	1·6	0·4–7·0	Undetermined	
Drank tap water	2·2	0·5–9·1	0·7	0·1–5·9
Travel	1·6	0·3–6·9	Undetermined	
Ate at restaurant	1·0	0·2–4·1	0·2	0·1–3·7
Family has garden	0·9	0·2–3·6	0·4	0·1–2·8
Visited cattle farms	2·8	0·4–15·8	Undetermined	
Visited chicken coops	6·5	1·2–34·9	6·0	0·5–64·7
Ate beef	0·2	0·1–1·5	Undetermined	
Ate veal	0·5	0·1–1·8	Undetermined	
Ate chicken meat	0·5	0·1–3·5	Undetermined	
Ate pork	0·7	0·2–2·2	0·2	0·1–1·7
Ate hamburgers	0·4	0·1–1·6	0·3	0·1–1·7
Drank unpasteurized milk	1·0	0·1–11·0	4·0	0·4–44·1
Ate unpasteurized cheese	1·0	0·1–5·4	2·0	0·1–31·9
Ate potatoes	0·2	0·1–2·7	Undetermined	
Ate raw vegetables	0·2	0·1–1·4	0·3	0·1–4·5

controls. The results are summarized in Table 1. The only risk factor significantly associated with the disease was the exposure of cases to chicken coops owned by their households.

Diarrhoea in households before the onset of HUS was present in one case and in none of controls. A restricted analysis for exposures for those infected with O157 demonstrated an OR of 6 for exposure to chicken coops, but the association was no longer statistically significant.

Other investigations

Examination of stool specimens obtained from 28 household contacts of 9 HUS cases revealed evidence of VTEC infection in 2 relatives. The grandmother of a child with serum antibodies to the O157 LPS was positive for free faecal VT1, and a VTEC belonging to an untypable serogroup was isolated from the grandfather of a case but with no evidence of VTEC infection. The serum of this case did not contain antibodies to the LPS of the VTEC strain from the grandfather.

The family of the case with *E. coli* O86 infection owned 2 calves, with which the child used to have close contacts. A third calf had been slaughtered 20 days before the onset of HUS. Two VTEC strains, both producing VT2 and belonging to untypable serogroups, but differing in their biochemical API profiles, were isolated from the stools of both animals.

Six out of the 9 chicken coops to which cases have been exposed were investigated. Thirty-eight faecal samples from chickens were cultured for VTEC with negative results. All the chicken owners had bought new chicks during spring 1993, before the beginning of the outbreak, but they were backtraced to different distributors. In addition, an attempt of tracing back to common feed wholesale distributors yielded no common source.

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With regard to beef consumption, 3 of the 15 cases were backtraced to a single wholesale beef distributor (2 who had antibodies to *E. coli* O157 and 1 to *E. coli* O111).

DISCUSSION

This outbreak represents the highest number of HUS cases ever described in Italy and Europe. The outbreak is also noteworthy in that it occurred over a large geographic area and over a several month period. Several features about this outbreak are of interest. First, the study confirms that outbreaks of VTEC infection which are not tightly clustered either geographically or temporally are difficult to detect in the absence of surveillance systems for VTEC or at least for HUS. No obvious epidemiologic link among the cases was observed, and also the clustering in space and time was loose. The sharp increase in the incidence of HUS when compared to the previous years and to the other Italian regions was the only evidence for the epidemic.

A second interesting feature is that it was not possible to identify patients with VTEC infection other than children notified to the HUS surveillance system. According to Griffin and Tauxe, outbreaks of *E. coli* O157:H7 infection usually involve adults and children, and only 2–7% of infected patients develop HUS [1]. Our investigation might not have been sensitive enough to detect cases other than children with HUS, and the outbreak could have potentially had larger dimensions. However, we do not believe that a large number of cases with severe bloody diarrhoea would have escaped our attention since the effort made by the hospital investigation and the communications given during paediatric meetings should have raised new cases if present. On the other hand, a high proportion of HUS cases among persons infected with VTEC is not unprecedented. Karmali and colleagues reported that in a family outbreak all the five siblings involved had HUS [17], and HUS cases accounted for 42% of the main cluster in a recent epidemic described in UK [10].

Evidence of infection with different serogroups was observed: 2 cases attributable to *E. coli* O111 lived about 5 km from each other and occurred in the first 2 weeks of May 1993; 8 more cases with evidence of infection by *E. coli* O157 occurred during summer and fall 1993 and were scattered in a wider area. Finally another child with clear exposure to calves, had microbiologic and serologic evidence of infection with *E. coli* O86.

The temporal and geographical scattering of cases, and the detection of at least three different VTEC serogroups, suggest that this outbreak could be ascribed to multiple sources or vehicles of infection that were, however, absent in previous years.

Despite the presence of diarrhoea in almost all the cases, and their young age, only 1 case had a family contact with diarrhoea before the onset of HUS and no secondary cases were detected in the households, although two asymptomatic relatives of the cases showed evidence of VTEC infection. This seems to exclude the possibilities that either person-to-person transmission or episodes of food poisoning may have played a major role in this outbreak as reported in other studies [1].

The case-control study did not show any significant association other than exposure to chicken coops. However, we failed either to isolate VTEC from chicken faeces or to trace back chicks to common wholesale distributors. Live poultry has never been identified as a reservoir of VTEC [18] even though *E. coli* O157:H7 has been isolated from retail poultry from grocery stores [19]. The possibility that chickens may host VTEC was suggested by Berry and colleagues [20] since the experimental infection of chicks with *E. coli* O157 resulted in a prolonged colonization of the caecum in absence of symptoms. On the other hand, we cannot exclude that the exposure to chicken coops could have been a chance finding or an indicator of exposure to other possible sources of infection not investigated in this study and also associated with living in rural areas. The choice of controls among acquaintances and neighbours of cases could have caused overmatching. This could have masked the association with the risk factors related to living in rural settlements considered in the study. By contrast, a control group randomly chosen among all residents in the whole region would have included more children living in large cities and it might have identified every exposure associated with rural living. In fact, 12 of the 15 cases (80%) involved in this outbreak lived in townships with less than 20000 inhabitants, compared with 54% of the general population in the three regions involved.

Interestingly, many outbreaks of *E. coli* O157 infection have been reported in rural settlements or in small townships [5, 7, 10, 11, 21], and one study on sporadic cases in central Europe [22] underlined an higher incidence of VTEC infection in rural areas. Moreover VTEC infection has been recently associated with eating vegetables from a manured garden [23] or drinking well water [21, 24]. In our study eight of the cases' families had a garden, but only two used to fertilize them with manure.

In conclusion, this outbreak confirms that VTEC infection is an emerging problem in Italy and epidemics with severe clinical manifestations can commonly occur. Only the presence of a surveillance system for HUS allowed us to recognize this outbreak that was not tightly clustered either geographically or temporally. Persons living in rural settlements seemed to be at a higher risk of acquiring VTEC infection in this outbreak, and more powerful studies are needed to identify the risk factors related to this lifestyle, including the exposure to live poultry.

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