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Sorbitol-fermenting enterohaemorrhagic *Escherichia coli* O157:H$^-$ causes another outbreak of haemolytic uraemic syndrome in children

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SUMMARY

An outbreak of haemolytic uraemic syndrome (HUS) among children caused by infection with sorbitol-fermenting enterohaemorrhagic *Escherichia coli* O157:H$^-$ (SF EHEC O157:H$^-$) occurred in Germany in 2002. This pathogen has caused several outbreaks so far, yet its reservoir and routes of transmission remain unknown. SF EHEC O157:H$^-$ is easily missed as most laboratory protocols target the more common sorbitol non-fermenting strains. We performed active case-finding, extensive exploratory interviews and a case-control study. Clinical and environmental samples were screened for SF EHEC O157:H$^-$ and the isolates were subtyped by pulsed-field gel electrophoresis. We identified 38 case-patients in 11 federal states. Four case-patients died during the acute phase (case-fatality ratio 11%). The case-control study could not identify a single vehicle or source. Further studies are necessary to identify the pathogen's reservoir(s). Stool samples of patients with HUS should be tested with an adequate microbiological set-up to quickly identify SF EHEC O157:H$^-$.

Key words: EHEC, foodborne infections, haemolytic uraemic syndrome, outbreak, sorbitol-fermenting *Escherichia coli* O157:H$^-$.

INTRODUCTION

Haemolytic uraemic syndrome (HUS) is defined as renal failure, thrombocytopenia and haemolytic anaemia and is the major cause of acute renal failure in childhood. Its primary cause is infection with enterohaemorrhagic *Escherichia coli* (EHEC), predominantly of serogroup O157 [1]. The inability to rapidly ferment sorbitol is a widely used screening criterion to differentiate this serogroup bacteriologically from other *E. coli*. However, a sorbitol-fermenting (SF), non-motile (H$^-$) EHEC O157 strain was first identified and characterized during an outbreak of HUS in Bavaria in 1988 [2]. Before autumn 2002, three

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outbreaks associated with SF EHEC O157:H$^-$ had been identified in Germany, all of which were initially recognized by an increase in the number of paediatric patients with HUS which was not paralleled by an increase of patients who experienced diarrhoea only ('HUS outbreak') [2–4]. Moreover, all three outbreaks occurred in colder months of the year, whereas most EHEC outbreaks occur during summer and autumn [5]. In only one of the outbreaks could a possible source of infection be identified [3]. A case-control study showed an association between illness and consumption of a raw spreadable sausage containing beef, but no microbiological confirmation was possible in the vehicle and trace-back to an animal reservoir was impossible. Whereas cattle have been well established as the major reservoir of EHEC O157:H7, to our knowledge, so far no animal reservoir has yet been identified for SF EHEC O157:H$^-$. Only rarely could SF EHEC O157:H$^-$ be isolated from animals: twice from cattle [6, 7] and once from a pony [8].

During 1997–2000, SF EHEC O157:H$^-$ were isolated from 21/207 (10%) EHEC-associated HUS patients during an active paediatric HUS surveillance conducted in Germany and Austria [9]. SF EHEC O157:H$^-$ have also been sporadically identified in several other European countries [7, 10–12], in Australia [13] and Asia [14], and recently caused simultaneous outbreaks in Scotland and northern England [15].

Several phenotypical and genetic features can be used to differentiate between SF and non-sorbitol-fermenting (NSF) EHEC O157, e.g. the lack of tellurite resistance and urease genes [16, 17] and the presence of the pilin subunit gene *sfpA* [18] in SF EHEC O157.

In Germany, both the clinical diagnosis of HUS for which an infectious aetiology cannot be ruled out and laboratory diagnosis of human EHEC infection have been notifiable diseases since 1998. Laboratory criteria for notification of EHEC infection are detection of Shiga toxin production in an *E. coli* isolate or amplification of Shiga toxin genes (*stx1* or *stx2*) from stool cultures [19]. In November 2002, six children who had HUS with disease onset in October were reported in Bavaria (compared with 10 HUS patients reported in Bavaria during the whole of 2001). Concomitantly, the National Consulting Laboratory on HUS (NCL-HUS) identified SF EHEC O157:H$^-$ in stools of 10 HUS patients in the course of 1 month. An investigation was initiated in collaboration with state and local health authorities to identify the outbreak's source, and risk factors for transmission.

METHODS

Case ascertainment and epidemiological investigations

To identify and investigate patients as quickly as possible, active case-finding was performed by contacting all paediatric nephrology units and reference laboratories in Germany. HUS was diagnosed clinically by paediatric nephrologists. We defined case-patients as children with onset of HUS between 1 October and 31 December 2002 (outbreak period) for whom infection with SF EHEC O157:H$^-$ could not be ruled out. Among them, confirmed cases were patients with culture-confirmed SF EHEC O157:H$^-$ infection, excluding HUS patients whose SF EHEC O157:H$^-$ isolates differed from the outbreak pattern in pulsed-field gel electrophoresis (PFGE) by two or more bands. Probable cases had only serological evidence of a recent *E. coli* O157 infection based on the detection of IgM antibodies against O157 lipopolysaccharide (LPS). Possible cases had no laboratory tests or results that did not definitely exclude an SF EHEC O157:H$^-$ infection.

After obtaining informed consent, the parents/guardians of case-patients were questioned through telephone interviews utilizing a 28-page specifically designed questionnaire concerning symptoms, food consumption, contact persons, travel history and contact with animals. In addition, a record of the daily activities and routines in the 4 days prior to symptom onset was obtained. As children with a migration background (i.e. cases or parents born outside Germany) appeared to be overrepresented among cases, households of four case-patients with a migration background from the state of Baden-Wuerttemberg were inspected regarding their living environment and a prevalence survey of food items present in the households was conducted. In early exploratory interviews, the consumption of apples picked up from the ground by individuals and of apple juice made from these apples was frequently reported. The juice was locally produced on a small scale and often unpasteurized. To test the hypothesis that disease was associated with consumption of this type of apple juice, we conducted a case-control study including case-patients with disease onset between 1 October and 10 December. Due to the limited number of cases, we included case-patients whose information originally had given rise to the hypothesis. Control individuals were randomly selected from notifications of infections with *Campylobacter* spp. and frequency-matched by federal state and age group.

The questionnaire asked about household size and distance to the next farm, travel, contact with animals, and about food consumed by the child and the child's household members. Particular emphasis was placed on consumption of home-made or locally produced apple juice, and consumption or collection of apples. Information was collected on several exposure periods including the week prior to disease onset (for case-patients) or interview (for controls) as well as the time since October 2002 and during the entire calendar year. Additionally, we asked about the frequency of consumption of these foodstuffs in the household. Data entry was performed with Epi Info version 6 (Epi Info™, CDC, Atlanta, GA, USA), bivariate analysis was performed using Epi Info software version 6 and version 2000. Exact confidence intervals (CI) were determined using Statcalc. Exact logistical regression was performed including possible risk-factors found in bivariate analysis to account for possible interaction, using Stata version 10 (command EXLOGISTIC; StataCorp, College Station, TX, USA) with federal state and age group as strata variables.

Microbiological and molecular investigations

Stool samples from HUS patients and their contacts were analysed at the NCL-HUS and the National Reference Centre for *Salmonella* and other enteric pathogens (NRC-Sal) for the presence of SF EHEC O157:H$^-$ using immunomagnetic separation, culture on sorbitol MacConkey agar (SMAC) and enterohaemolysin agar and polymerase chain reaction (PCR) screening of the primary stool cultures for *stx*, *eae*, rfb_{O157}, and *sfp*A as described previously [18]. Isolates were subtyped by PFGE following the PulseNet protocol of CDC, Atlanta [20] and the PFGE patterns were analysed with BioNumerics, version 4.5 (Applied Maths BVBA, Belgium) at the NRC-Sal. If available, the sera of HUS patients were screened for IgM and IgG antibodies to O157 LPS [21, 22].

Environmental and contact investigations

Samples obtained from the environment and contacts of the patients were analysed by PCR for the presence of rfb_{O157} and *stx* genes [18], and for the production of Shiga toxin (Stx) by an in-house Gb3-receptor enzyme immunoassay in the Veterinary National Reference laboratory for *E. coli* in Dessau, Germany.

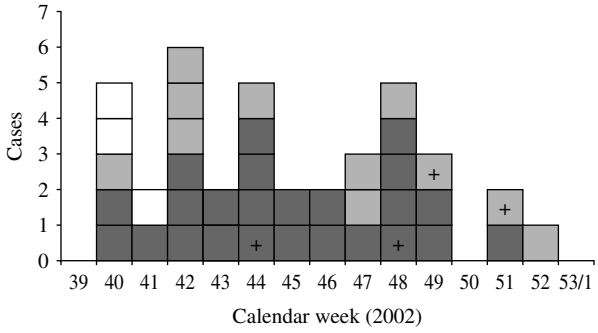

Fig. 1. Possible (□), probable (▨) and confirmed (■) case-patients in an outbreak of haemolytic uraemic syndrome due to sorbitol-fermenting EHEC O157:H$^-$ in Germany by week of onset, October–December 2002 ($n=38$). +, Fatal case.

RESULTS

Case ascertainment and epidemiological investigations

We identified 48 paediatric HUS patients during the outbreak period. Of these, 38 children met the definition for cases related to this outbreak (Fig. 1): we classified 22 as confirmed cases, 13 as probable and three as possible cases. Ten HUS patients were considered unrelated to this outbreak. In stool samples of five of these patients, SF EHEC O157:H$^-$ were isolated which differed in the PFGE pattern by two or more bands from the outbreak strain; of these, two SF EHEC O157:H$^-$ strains were *stx*-negative. From five other HUS patients, a non-O157 EHEC was isolated. A total of four (two confirmed, two probable) case-patients died during the acute phase of the disease, resulting in a case-fatality ratio of 11% (95% CI 4–26) for confirmed and probable cases combined.

The median age of the 22 confirmed case-patients was 2·7 years (range 1–9 years); 11 were males. Nineteen (86%) lived in the two southern German states of Bavaria and Baden-Wuerttemberg (Fig. 2). Seven (32%) of the confirmed case-patients had a migrant background [the cases themselves or their parents originated from countries of the former Soviet Union (3x), Turkey (2x), and Kosovo (1x); one declined to specify]. Case-patients without a migrant background were more likely to live in rural areas. None of the case-patients lived in a town with more than 50 000 inhabitants, compared to 22% of families with children aged <15 years in the general population according to 2001 microcensus data of the German Federal Statistical Office ($P=0{\cdot}02$). Inspection of the living environment of four households of case-patients with migrant background did not

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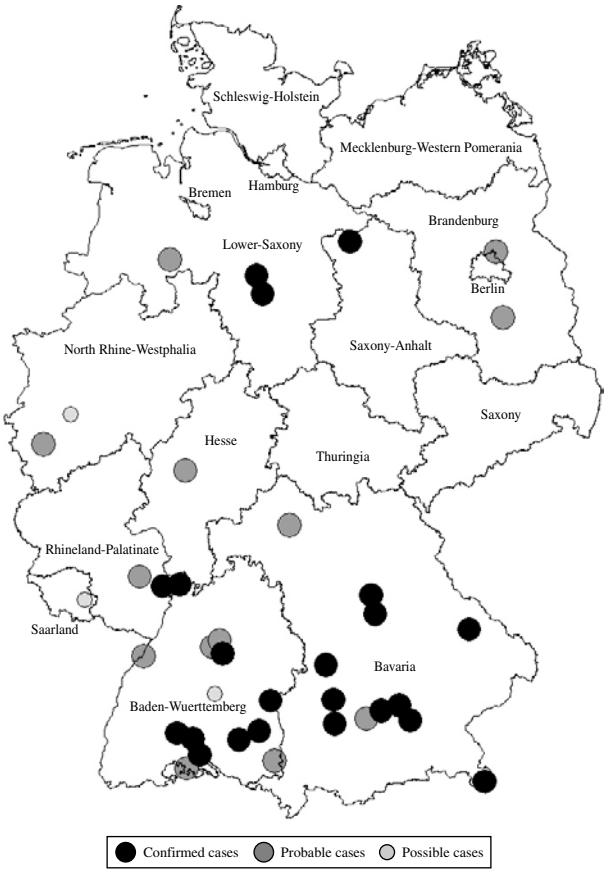

Fig. 2. Possible, probable and confirmed case-patients in an outbreak of haemolytic uraemic syndrome due to sorbitol-fermenting EHEC O157:H$^-$ in Germany by place of residence, October–December 2002 ($n=38$).

identify a commonality. In the food prevalence survey, food items of the same brand were never found in more than two households.

In the case-control study, data from 16 case-patients (11 confirmed, 5 possible) and 67 controls were analysed. Self-reporting of the case-patients revealed that 11 (67%) had vomited, and 12 (75%) had had diarrhoea (among them seven with bloody diarrhoea). All children were admitted to hospital with a median duration of 34 days of hospitalization (range 7–42, number of days missing for one child, four children were still in the hospital at the time of the interview).

Case-patients and controls lived predominantly in rural areas of Germany. Moreover, 8/11 (72%) case-patients and 42/61 (69%) controls for whom we could obtain the information lived within 1 km of a farm; five controls (7·5%) and no case-patient lived on a farm.

In bivariate analysis, the occurrence of HUS was associated with the consumption of apple juice [odds ratio (OR) 6·6, 95% exact CI 1·5–27·45] (one control excluded due to a 'don't know' answer). However, the information available indicated that the apples used in preparing the apple juice were obtained from various orchards and the juice was produced by different manufacturers. HUS was also significantly associated with a type of fruit-laced and sweetened 'quark' product popular with children in Germany (OR 4·8, 95% exact CI 1·3–19·6). Quark is a fresh curdled cheese akin to farmer's cheese produced from pasteurized milk by fermentation with lactobacilli and/or renin. After fermentation the whey is removed and cream, sugar and fruit are added. A variety of different brands were mentioned for this type of quark preparation, which is packaged like small servings of yoghurt. Five cases (31%) *vs.* four controls (6%) had consumed apple juice as well as quark. The results of exact conditional logistic regression suggest that both risk factors are not independently associated with infection (Table 1).

None of the cases had consumed raw milk *vs.* 12 controls (18%) who had. Consumption of sausages, minced meat or salad was not found to be associated with infection.

Environmental and contact investigations

EHEC was not isolated from apples from case-patients' households, apple juice and environmental samples (soil, leaves, sand and dog faeces); all also tested negative for EHEC in the enzyme immunoassay and PCR tests.

In only three of the infected patients (19%), did the parent/guardian mention having had diarrhoea in the week preceding the onset of symptoms in the child. Contact investigations were incomplete, but in Bavaria only three EHEC-positive stools were found in contacts to four confirmed cases-patients (total number tested not known). During the outbreak period, there was no corresponding increase in notifiable EHEC infections: between October and December 2002, 340 EHEC infections were notified in Germany compared to 305 between October and December 2002 (http://www3.rki.de/SurvStat/).

DISCUSSION

We report the largest known outbreak of paediatric HUS due to SF EHEC O157:H$^-$ thus far. It was characterized by a high case-fatality ratio and the absence of a recognized increase of EHEC-associated gastroenteritis without HUS. Occurrence of cases

Table 1. *Risk factors for HUS in an outbreak of HUS due to sorbitol-fermenting EHEC O157:H⁻ in Germany, 2002*

Exposure	Cases ($n=16$)	Controls ($n=67$)	aOR	95% CI
Consumption of locally produced apple juice	7 (44%)	7 (11%)	8·8	0·6–116·4
Consumption of 'quark'	11 (69%)	21 (31%)	4·7	0·8–35·3
Interaction Apple juice * quark	5 (31%)	4 (6%)	0·4	0·0–11·0

HUS, Haemolytic uraemic syndrome; aOR, adjusted odds ratio; CI, confidence interval.

appeared to be geographically dispersed and protracted. Case-patients lived predominantly in rural areas.

Despite intense efforts, which included exhaustive questioning of members of case-patients' families and a food prevalence survey in selected households, we were unable to identify a common source for this outbreak. The case-control study suggested a statistical association of illness with having consumed locally produced apple juice and a quark product. The statistical analysis could not resolve which of the two may have been the vehicle of infection. Unpasteurized apple juice has been implicated in several outbreaks caused by EHEC O157:H7 to the acid tolerance of these organisms [23]. Thus, apple juice would have been a plausible vehicle. However, the consumption of apple juice was reported by only 44% of the cases and apples consumed by the patients were not obtained from a common collection or production site. Furthermore, cases continued to occur after completion of the case-control study, and most of these later case-patients denied having consumed locally produced apple juice. Moreover, only one of the case-patients with a migrant background reported consumption of apple juice. Consumption of locally produced apple juice may have been a surrogate factor for a rural exposure or season of the year, because apple picking and delivery to local manufacturers are autumn activities and apples are typically harvested in rural areas. However, controls, randomly selected among children with notified *Campylobacter* spp. infection, were similar to the case-patients in the sense that they were also predominantly rural. Thus, they should have had similar opportunities to consume locally produced apple juice. Regarding the association of the quark product and disease, a causal role is rendered unlikely by the broad variety of brands involved. However, it was not possible to trace back all ingredients to their origins. Hence, we cannot exclude that one of the components of the quark stemmed from the same production source and may have had some causal relation with the infection or was associated with the true risk factor.

The case occurrence in this and also in previous German SF EHEC O157:H⁻ outbreaks seems to point to a distinct epidemiology of human infection associated with these strains that differs from those caused by NSF EHEC O157. The protracted and widespread distribution of cases is compatible with a widely distributed food vehicle with a long shelf-life or that had been repeatedly contaminated. Reasons for our inability to identify a common vehicle may lie in incomplete recording of case-patients' food history, incomplete recall of parents/guardians (e.g. ubiquitous vehicles such as some spices), existence of more than one contaminated vehicle or transmission route, or a combination of these factors. Alternatively, the repeated failure to identify a common food vehicle, despite extensive evaluation of food history, may indicate an unrecognized environmental risk rather than a foodborne risk.

The scattered distribution and the absence of a corresponding increase in non-HUS SF EHEC O157:H⁻ infections raises the question of whether there might be a genetic or other predisposing factor for HUS once infected with this specific strain. An association between blood group and HUS has already been suggested [24]. The role of a genetic factor would also fit the observation of a higher than representative proportion of case-patients with a migrant background, as the genetic pool of migrant populations may be different from the majority population. In the states of former West Germany, 21% of the 2005 population had a migrant background, compared to 32% of the confirmed cases ($P=0·3$). Region-specific information for the state or area where the outbreak took place is not available.

As the outbreak affected mainly rural areas, the proportion of inhabitants with a migrant background can be assumed to be lower than the West German average because urban areas tend to have higher proportions of migrant population. Alternatively, SF EHEC O157:H$^-$ could possess virulence determinants not present in NSF EHEC O157 in addition to *stx2* and *eae*, which are considered the major virulence factors of EHEC [25–27]. In an outbreak of SF EHEC O157:H$^-$ infection in Scotland, 17 cases with diarrhoea were identified and of these, eight (47%) progressed to HUS [28]. During a 10-year period, we followed 18 sporadic patients with diarrhoea caused by SF EHEC O157:H$^-$ and of these 12 (66·6%) developed HUS (H. Karch, personal communication). These independent findings may indicate an even higher virulence of SF EHEC O157:H$^-$ than that of NSF EHEC O157.

This outbreak could only be detected because the stool samples were cultured on tellurite-free media, which allow growth of tellurite-sensitive SF EHEC O157 [17]. By using cefixime-tellurite sorbitol MacConkey agar, which is a common selective medium for the isolation of tellurite-resistant NSF EHEC O157:H7 this outbreak would have been missed. By applying conventional criteria to interpret PFGE patterns with regard to outbreak relatedness, we excluded almost as many HUS patients with SF EHEC O157:H$^-$ as are usually identified during a whole year. However, subsequently to the investigation of this outbreak, we have recently shown that PFGE patterns of SF EHEC O157:H$^-$ can change during the course of an outbreak [29] and that strains can loose their *stx* genes [22, 29]. Therefore, it is possible that some of the excluded patients were misclassified and were actually part of the outbreak.

We conclude that SF EHEC O157:H$^-$ probably have a higher virulence than NSF EHEC O157 and have the potential to cause large HUS outbreaks. The apparently high virulence of SF EHEC O157:H$^-$ necessitates the need to urgently identify the pathogen's reservoir(s) and vehicles of infection, and to determine whether there are genetic or other predisposing factors for infection with this strain. For a more accurate exposure assessment of SF EHEC O157:H$^-$ infected patients, rapid identification of these patients is crucial. Therefore, continued enhanced surveillance of HUS is needed in combination with an adequate microbiological set-up to quickly recognize SF EHEC O157:H$^-$. This pathogen is easily missed as most laboratory protocols target the more common sorbitol non-fermenting strains. Stool samples of HUS patients should be tested by culture on tellurite-free media, immunomagnetic separation or nucleid acid-based methods. Future outbreaks as well as sporadic patients with an infection with this pathogen should be expediently investigated, including a more thorough exploration of possible environmental transmission routes.

Considering the findings of this outbreak, in keeping with the results of a case-control study of risk factors for sporadic EHEC-associated illness in Germany [30], future outbreak investigations should place emphasis on extensive recording of outdoor activities, and outbreak investigations should also include screening of household contacts for possible SF EHEC O157:H$^-$ carriage.

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DECLARATION OF INTEREST

None.

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