

Surveillance and outbreak reports

OUTBREAK OF VEROCYTOTOXIN-PRODUCING *E. COLI* O145 AND O26 INFECTIONS ASSOCIATED WITH THE CONSUMPTION OF ICE CREAM PRODUCED AT A FARM, BELGIUM, 2007

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In October 2007, an outbreak of verocytotoxin-producing *Escherichia coli* (VTEC) O145 and *E. coli* O26 occurred among consumers of ice cream produced and sold in September 2007 at a farm in the province of Antwerp (Belgium). The ice cream was consumed at two birthday parties and also eaten at the farm. Five children, aged between two and 11 years, developed haemolytic uraemic syndrome (HUS), and seven other co-exposed persons contracted severe diarrhoea. In three of the five HUS cases VTEC O145 infections were laboratory confirmed, one in association with VTEC O26. Identical isolates of *E. coli* O145 and O26 were detected with PCR and PFGE in faecal samples of patients and in ice cream leftovers from one of the birthday parties, in faecal samples taken from calves, and in samples of soiled straw from the farm at which the ice cream was produced. Ice cream was made from pasteurised milk and most likely contaminated by one of food handlers.

Introduction

Verocytotoxin-producing *Escherichia coli* (VTEC), including *E. coli* O157:H7, O26, O145 and other *E. coli* serotypes, are important causes of gastrointestinal illness and haemolytic uraemic syndrome (HUS) in young children. This syndrome is characterised by haemolytic anaemia, thrombocytopenia and acute renal failure, a complication occurring in 5-14% of VTEC infections [1,2]. HUS is a potential life-threatening disease and can induce hypertension, proteinuria and chronic renal failure in 5% of affected patients. The age group primarily affected are children under five years. VTEC O157:H7 is considered as the most clinically significant serotype, and is often associated with severe bloody diarrhoea and HUS. The prevalence of VTEC serotype O145 in human infections is relatively low, accounting for 5%-7% of all non O157 strains in prevalence studies [3]. The range of products associated with VTEC infections is wide: hamburger, ground beef, cider, spinach, unpasteurised ice cream, milk and cheese, and others. Infections have also been linked to municipal water supplies [4,5].

VTEC infections in Europe and the United States have increased in the last decade causing several large epidemics of food poisoning in industrialised countries [4,5,6]. In Belgium, however, it has

been a sporadic disease [7,8]. In 2005, the incidence of VTEC in Belgium was 0.5 cases per 100,000 population, compared to a mean incidence in Europe of 1.2 cases per 100,000 population [7,8,9]. However, this is probably an underestimation, as most of the country's clinical laboratories do not test for these microorganisms in routine gastroenteritis samples [8].

We report on five children with HUS of which three had a laboratory confirmed VTEC O145 infection. All patients consumed ice cream produced and sold at a farm.

Methods

The outbreak

On 2 October 2007, the detection of three isolates of VTEC O145, one of which was associated with *E. coli* O26, was reported by Belgium's Federal Reference Laboratory for *E. coli* to the Antwerp department of Infectious Diseases Control. The strains were obtained from patients hospitalised with HUS and living in the northern part of Antwerp province. On 3 October, investigators instituted active case finding and interviewed the parents of the different patients. All patients had eaten ice cream produced and sold at the same farm within eight days of developing gastrointestinal symptoms. The ice cream was consumed at two birthday parties or consumed on the farm.

The farm was a traditional dairy farm with a limited number of cows, young cows and calves. The farm that made the ice cream was well known in the region and, depending on season and weather, up to 160 litres of ice cream were sold daily.

On 3 October, the Antwerp Department of Control of Infectious Diseases invited the Antwerp Department of the Federal Agency for the Safety of the Food Chain, the Laboratory of Food Microbiology of the University of Ghent, and the Reference Laboratory for *E. coli* of the University of Brussels to assist in the investigation. The study was carried out to determine the impact of the outbreak, to identify risk factors, and to interrupt transmission. On 5 October, investigators were informed of the existence of leftovers of ice cream consumed at one of the birthday parties.

Epidemiologic investigation

In order to develop hypotheses regarding possible sources of *E. coli* O145 infections investigators interviewed the parents of the patients who contracted HUS on 3 October using an adapted standard questionnaire for HUS' investigation generated by the Netherlands' Landelijke Coördinatiestructuur Infectieziektebestrijding (Coordination Structure for Combating Infectious Diseases) [10]. They were asked about consumption of food and drinks, contact with animals (domestic, farm, zoo), and travel history in the 10 days before onset of diarrhoea.

Hypothesis – generating interviews suggested that the outbreak occurred among participants in two birthday parties with eight and 11 participants respectively, respectively on 14 and 16 September. A third group of consumers was an undefined group of individuals who consumed ice cream at the farm. Consequently a retrospective cohort study was conducted among the participants in the birthday parties.

For case-ascertainment purposes, a probable case of HUS was defined as a patient who developed acute diarrhoea (three or more loose stools in a 24-hour period) complicated with HUS (acute haemolytic anaemia, thrombocytopenia, and signs of renal failure) occurring within 10 days of consumption of ice cream produced at a farm in Mol in September 2007. A confirmed case with HUS was defined as a patient meeting the criteria of a probable case and accompanied by isolation of *E. coli* O145 and/or *E. coli* O26 in stools or a positive serology for *E. coli* O145 and/or O26. A patient with a probable VTEC diarrhoea infection without HUS was defined as a patient who developed acute diarrhoea (three or more loose stools in a 24-hour period) in the 10 days following consumption of the farm-made ice cream and the patient belonged to a group in which a confirmed case has been detected. A patient with only a confirmed VTEC infection was a patient meeting the criteria of a probable VTEC diarrhoea infection accompanied by *E. coli* O145 and/or *E. coli* O26 in stools or a positive serology for *E. coli* O145 and/or O26.

A retrospective cohort study was established among the participants in the birthday parties. Relative risks and P-values (Fisher exact) were calculated using Epi Info, version 3.3.2 [11].

Environmental investigation

The farm's layout, ice cream production process and staff activities were determined. Different environmental samples were obtained: faecal samples from animals (calves, young animals and cows), and samples from each pen floor, dust and feed. To identify the source of the infection, raw milk, fresh ice cream produced at the farm and leftover portions of the ice cream from the birthday party on 16 September 2007 were sampled.

Microbiological examination and molecular analysis

Stools and urine samples of HUS patients were collected, and sent to the Belgian reference laboratory for *E. coli* for microbiological analysis. The stools were cultured using SMAC/SMAC+CT medium. On the basis of biochemical tests, PCR, and agglutination assay VTEC of serogroup O145 and O26 were identified. Additional PCR tests were performed to identify specific virulence genes carried by these VTEC strains. Serum samples of the fifth HUS patient were collected and tested for presence of anti-VTEC antibodies using agglutination assay.

Pulsed Field Gel Electrophoresis (PFGE) was used to examine and to compare the genetic profiles of the VTEC isolates.

Results

Clinical information and epidemiologic information

By 5 October, five cases of HUS had been identified among consumers of ice cream sold at the farm between September 12 and 16, 2007 (Figure 1). All patients with HUS were girls aged between two and 11 years. Three cases met the criteria of a confirmed case. VTEC of serogroups O145 and O26 were isolated from faeces and urine in a two-year-old girl and serogroup O145 was isolated from the faeces of an eight-year-old girl. In a third case, serology was

FIGURE 1
Cases of haemolytic uraemic syndrome (HUS) and VTEC diarrhoea by onset of symptoms, Mol, Belgium, 2007

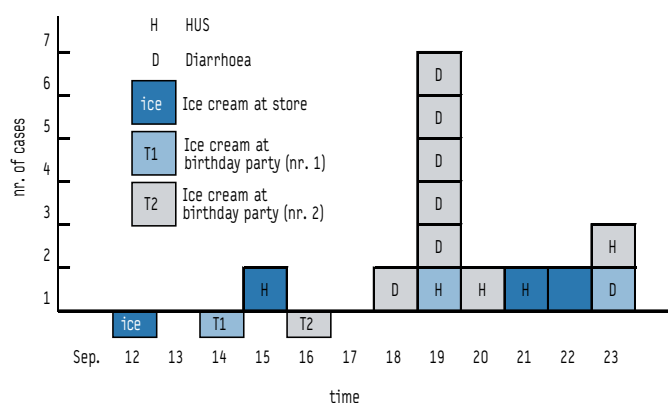
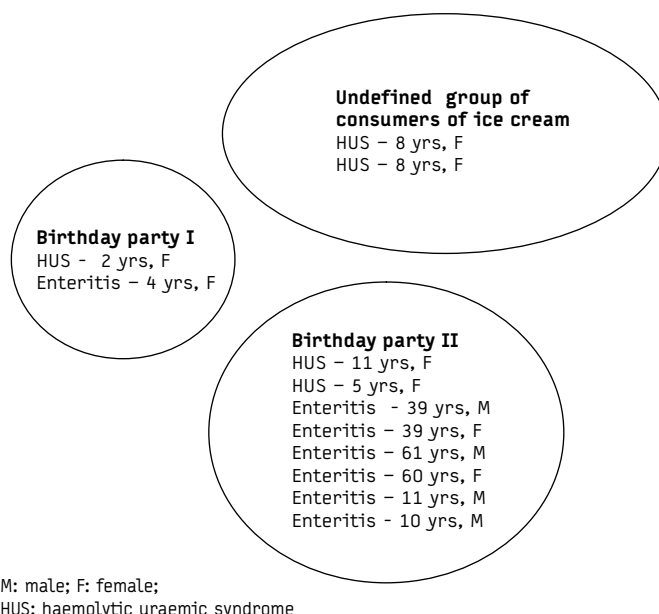


FIGURE 2
Cases by place of consumption of ice cream, VTEC outbreak, Mol, Belgium, September 2007



positive for O145 antigen. All HUS patients were admitted to the hospital, two requiring haemodialysis and three transfusion. No deaths have occurred among the identified patients

Two of the cases had eaten ice cream at the farm and three cases during birthday parties. Seven cases of acute diarrhoea were identified among persons co-exposed at the same birthday parties. The age of the diarrhoea patients ranged from four to 61 years.

The distribution of the HUS cases and the VTEC diarrhoea by time and place of consumption of the ice cream is shown in Figures 1 and 2. The mean incubation period between infection and onset of diarrhoea was five days. The mean interval between onset of diarrhoea and HUS was 5.6 days.

Of all the VTEC cases, only two patients had visited the farm but had had no contact with the stables or animals. No cases of VTEC diarrhoea could be identified among the consumers of ice cream at the farm.

The attack rate for patients with HUS among the participants in Birthday Party 1 on 14 September was 12.5% (1/8) and 18% (2/11) in Birthday Party 2 on 16 September. The attack rate for the probable VTEC infections was 25% (2/8) in Birthday Party 1 and 73% (8/11) in Birthday Party 2. No diarrhoeal illness was reported among the farm workers and the staff involved in the ice cream preparation. Relative risks were undefined in Birthday Party 1 and 2. P-values calculated with Fisher exact one tailed test were $p=0.75$ in Birthday Party 1 and $p=0.20$ in Birthday Party 2.

Environmental study and microbiologic data

Evaluation of the ice cream production process did not reveal major processing errors. Pasteurised milk was used for the production of the ice cream. One person who was normally not involved in the production process of ice cream and who also worked at the farm participated only in the production of ice cream in the week of 12 September. Fresh milk stored at the farm and prepared ice cream samples collected on 4 October 2007 were negative for VTEC O145 and O26 pathogenic bacteria, but faecal samples of calves and dust samples of the calves' stables were positive for VTEC O145 and O26. Leftovers of the ice cream consumed at Birthday Party 2 on 16 September were also positive for VTEC O145 and O26.

Molecular analysis

Sorbitol-fermenting VTEC O145 strains were identified in stools of two HUS patients, one in association with serogroup O26 which was isolated in both stool and urine samples. PCR analysis revealed that the VTEC O145 and O26 isolates were positive for, respectively, verocytotoxin type 2 and type 1 (VT2 and VT1). Both serogroups were positive for additional virulence genes *eaeA* and *ehxA*. Agglutination assay performed on the serum samples of the fifth HUS patient revealed the presence of anti-VTEC O145 antibodies.

PFGE was performed on the VTEC strains isolated from patients, ice cream, and the farm environment. These results confirmed that the VTEC O145 strains, isolated from the two female patients were undistinguishable from isolates from ice cream and samples collected on the farm (Figure 3.A, lanes 3, 4, 6, 7, 8, and 9). The VTEC O26, isolated from faeces and urine of a two-year-old female patient, were undistinguishable from VTEC O26 isolated from the environment of the farm and the ice cream (Figure 3.B lanes 3, 4, 6, and 7).

Discussion

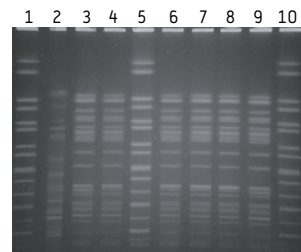
We have described an outbreak of VTEC infections among individuals who had eaten ice cream produced and sold at a farm in the northern part of the province of Antwerp. The infections were transmitted from animals and their environment to people.

There is strong epidemiological evidence to assume that the incriminated vehicle was contaminated ice cream. All patients had eaten ice cream sold at the farm in the week before onset of the diarrhoea and HUS. Individuals participating in the birthday parties where the ice cream was eaten and who had never been at the farm, developed the disease. Most likely due to the small number of participants in the parties, a significant association among the ice cream eaters and the disease could not be identified. The leftovers of the ice cream and the stools of the two patients with positive cultures had identical PFGE profiles for VTEC O145 and O26 respectively. Taking into account that pasteurised milk was used in the production of the ice cream, cross-contamination is the most likely explanation for the contamination. One of the most likely explanations might be the participation in the production process of an individual who was not trained, properly instructed and had contact with the animals.

FIGURE 3

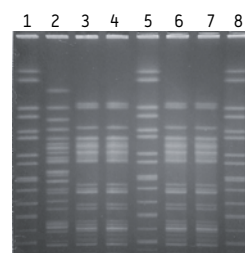
Molecular typing of VTEC isolates, Mol, Belgium, September 2007

(A) PFGE analysis of VTEC O145 isolates



Lane 1: *Salmonella Braenderup* H9812 (molecular size standard), lane 2: unrelated VTEC O145 isolate, lane 3: VTEC O145 isolate in stool of a female patient aged 8 years, lane 4: VTEC O145 isolate in stool of a female patient aged 2 years, lane 5: *S. Braenderup*, lane 6: VTEC O145 isolate from hay sample of the farm, lane 7: VTEC O145 isolate from dust sample of the barn, lane 8: VTEC O145 isolate from faeces of calf, lane 9: VTEC O145 isolate from ice cream cake, lane 10: *S. Braenderup*.

(B) PFGE analysis of VTEC O26 isolates



Lane 1: *S. Braenderup*, lane 2: unrelated VTEC O26 isolate, lane 3: VTEC O26 isolate in stool of a female patient aged 2 years, lane 4: VTEC O26 isolate in urine of female patient aged 2 years, lane 5: *S. Braenderup*, lane 6: VTEC O26 isolate from ice cream cake, lane 7: VTEC O26 isolate from environment of calf stable, lane 8: *S. Braenderup*.

Only the five HUS cases were tested for non-O157 VTEC and of these only three were confirmed as VTEC infections. The VTEC diarrhoea cases were not confirmed by laboratory testing. Testing of faecal samples of patients with diarrhoea for the presence of VTEC is not regularly undertaken in Belgium. One of the reasons might be that testing for *E. coli* is not reimbursed by social security in Belgium. The delay between acute diarrhoea and the onset of HUS, late diagnosis and the intake of antibiotics before diagnosis might also explain the absence of VTEC confirmation in two of the HUS patients.

To our knowledge, this is the first outbreak of HUS and VTEC caused by O145 and O26 in Belgium associated with the consumption of ice cream made from pasteurised milk. Outbreaks associated with VTEC O157 among visitors to a dairy farm were recently described in Belgium [12], with consumption of unpasteurised milk as a source of the VTEC, as described by Allerberger *et al.* [13].

There is significant morbidity and mortality associated with diarrhoea-associated HUS in children due to the devastating microvascular thrombotic angiopathy [1,2]. A Canadian prospective study showed an annual incidence of 1.11 case of diarrhoea associated HUS per 100,000 children under the age of 16 years [14]. The disease occurred most frequently in children younger than five years old [15]. However, in this outbreak, only one out of five patients belonged to this age group. This probably underscores the underdetection of HUS in the population.

Faecal samples of calves and dust of the barn were positive for VTEC O145 and O26. Studies on prevalence of *E. coli* O157 in cattle in Belgium show percentages ranging from 0 to 85% according to age of animals, specific farms, herds, and time of sampling. Young animals in particular have higher carriage. The prevalence of *E. coli* O157:H7 in beef carcasses was 1.1% (N=2,554) in 2005, while no VTEC were detected in 175 samples of raw milk in 2005 [7]. No data are yet available regarding the prevalence of other serogroups in Belgian cattle.

This outbreak underscores the need to consider zoonotic transmission and to highlight the prevention measures in facilities where there is easy contact with farm animals and their environment. Moreover, in our case the presence of VTEC in cattle at the farm and the shared activities of food-handling are problematic, as these pathogens can survive for months on surfaces [3].

The association between ice cream made with pasteurised milk and VTEC is very unusual [13,14]. However as shown in this outbreak, cross-contamination is a significant risk. Our data underline the need to reinforce hygienic measures for food-handlers working at farms where food products are prepared.

This study illustrates the usefulness of appropriate source tracing in VTEC infections and possibilities of good collaboration among the clinicians, microbiologists, and public health officials.

Control measures

To control the outbreak GPs, paediatricians, hospitals and health authorities were alerted and asked to look for cases. The food-preparing process and the quality of the ice cream and milk were checked and identified as free of VTEC. The food handlers were informed about the risk of contamination and prevention. Finally, the Safety in the Food Chain Agency is considering launching a prevention campaign targeted at this kind of facility.

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