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ORIGINAL ARTICLES

Outbreak report

FIRST GENERAL OUTBREAK OF VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* O157 IN DENMARK

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This report describes the first general outbreak of verocytotoxin-producing *E. coli* (VTEC) in Denmark. Twenty five patients, 18 children and seven adults, with culture-confirmed VTEC O157:H- infection and indistinguishable pulsed-field gel electrophoresis DNA profiles, were identified during a six month period from September 2003 to March 2004. The outbreak strain possessed the virulence genes: *eae*, *vtx1* and *vtx2c*. All patients but one presented with diarrhoea; none developed haemolytic uraemic syndrome. The outbreak was restricted to Copenhagen and surrounding areas. A case-control study including 11 cases and 55 matched controls revealed an association between VTEC O157:H- infection and shopping in a specific supermarket chain in Copenhagen and surrounding area, matched odds ratio (OR): 8.7 (95% confidence interval (CI): 1.1-71). After exclusion of three assumed secondary cases, only consumption of a particular kind of organic milk from a small dairy was associated with disease OR: 8.7 (95% CI 1.6-48). Environmental and microbiological investigations at the suspected dairy did not confirm the presence of the outbreak strain, but the outbreak stopped once the dairy was closed and thoroughly cleaned.

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Key words: *Escherichia coli* O157; VTEC; outbreak; milk; diarrhoea; Denmark

Introduction

Verocytotoxin-producing *Escherichia coli* (VTEC) is an important cause of gastroenteritis, in particular in industrialised countries [1,2]. In recent decades, VTEC has caused a number of outbreaks affecting large numbers of people [3,8], including outbreaks associated with both pasteurised and unpasteurised milk [9,12].

VTEC is mandatorily reportable in Denmark both through laboratory based surveillance and clinical notifications from the treating physician. Based on laboratory reports, the incidence has increased from 1.0 per 100 000 population in 1999 (53 cases), to 3.1 per 100 000 in 2004 (168 cases) [13,15]. This trend is most likely due to an increased number of stool specimens examined for diarrhoeagenic *E. coli*, including VTEC. General outbreaks of VTEC gastroenteritis have not previously been seen in Denmark; only sporadic cases or small family clusters of infection have been detected [13].

In late 2003, the Danish VTEC reference laboratory at Statens Serum Institut observed that seven isolates of VTEC O157:H- had identical patterns as judged by pulsed-field gel electrophoresis. The samples were received over a period of four months. In January and February 2004, seven additional isolates were detected, and we initiated an investigation of this first general outbreak of VTEC infection in Denmark. The objectives of the investigation were to characterise the outbreak and, if possible, determine the vehicle.

Methods

Diagnostics and surveillance

Diagnostics for VTEC infection are carried out either at the Unit of Gastrointestinal Infections (UGI), Statens Serum Institut (SSI), or at regional clinical microbiology laboratories (CMLs). UGI receives stool samples for diagnostics of VTEC and other bacterial enteropathogens as well as VTEC strains isolated at CMLs. Examination for diarrhoeagenic *E. coli* at UGI was performed by plating on SSI enteric medium [16] followed by a multiplex PCR determining the presence of the following virulence genes: Genes encoding for verocytotoxin (*vtx1* and *vtx2*), the toxins of enterotoxigenic *E. coli* (*elt*, *estA*), intimin (*eae*), a marker of enteroinvasive *E. coli* (*ipaH*), and with 16S rDNA serving as a positive control. All VTEC isolates were thoroughly characterised with determination of virulence factors [17,18] and DNA-profiles by pulsed-field gel electrophoresis (PFGE) typing [19]. O:H serotyping was performed according to the methods described by Ørskov and Ørskov, using SSI diagnostic antisera [20]. Selected strains were phage typed at the Laboratory of Enteric Pathogens at the Health Protection Agency Centre for Infections, United Kingdom (21).

Case-control study

We conducted a case-control study from 17 to 25 March 2004. Cases were defined as patients with a laboratory confirmed VTEC O157:H- infection of the PFGE outbreak-type, diagnosed after 15 January 2004. Based on hypothesis-generating interviews, a six page case-control questionnaire was prepared. It included questions on clinical and demographic data as well as exposure variables, e.g., contact with animals, shopping preferences, consumption of organic foods, meat and meat products (including sausages), juice, fresh produce, dried fruits, and dairy products. Controls selected from the Danish population registry were matched to patients by date of birth, sex, and post code of residence but were otherwise randomly chosen. Participants were interviewed by telephone. The period under study was the week before symptom onset for cases and the week before interview for controls. Controls were excluded if they had experienced diarrhoea during the study period. Six controls per case were interviewed. Data were analysed by conditional logistic regression using Statistical Analysis System (SAS) version 8.2; matched odds ratio (OR) and 95% confidence intervals were calculated.

Environmental investigations

Regional food control officers carried out environmental investigations at a dairy where a suspected food item had been processed. The investigations included inspection of the production site, review of all log books, and collection of samples (raw milk, pasteurisation unit, processing system, packed milk and sewage drains). All samples were examined for VTEC O157 by the standard methods recommended by the Nordisk Metodikkomite for Næringsmidler (Nordic Committee on Food Analysis, <http://www.nmkl.org>).

Results

Characterisation of the outbreak strain

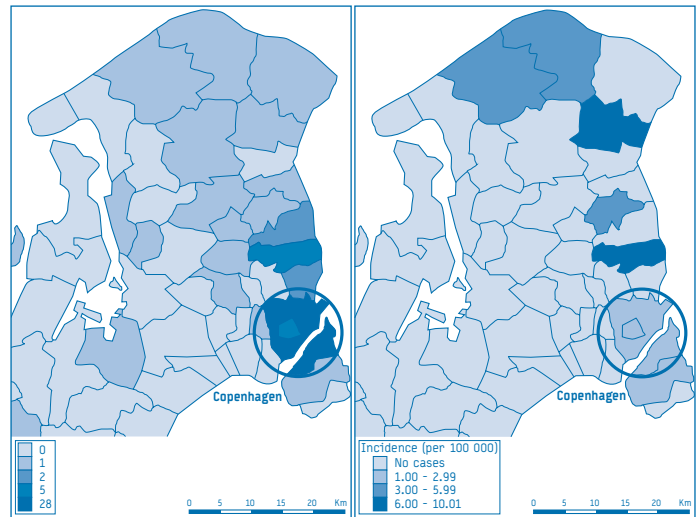
Between September 2003 to March 2004, 25 strains of VTEC O157:H- were isolated. All strains had a unique PFGE type and the virulence profile: *eae*, *vtx1* and *vtx2c*. Three strains were selected for phage typing and found to be phage type 8.

Descriptive epidemiology

Geographically, patients were restricted to Copenhagen and its northern suburbs [FIGURE 1]. Among the 25 patients [FIGURE 2], the sex ratio (female/male) was 19/6. In total, 18 were children aged 1-7 years and seven were adults aged 36-60 years. Person-to-person transmission could not be excluded in some patients; three families had two patients each and two daycare institutions had two and three patients, respectively. All patients experienced relatively mild illness; the predominant symptoms were diarrhoea and abdominal cramps, and at least five had bloody diarrhoea. Two patients were admitted to hospital, but none developed haemolytic uraemic syndrome.

FIGURE 1

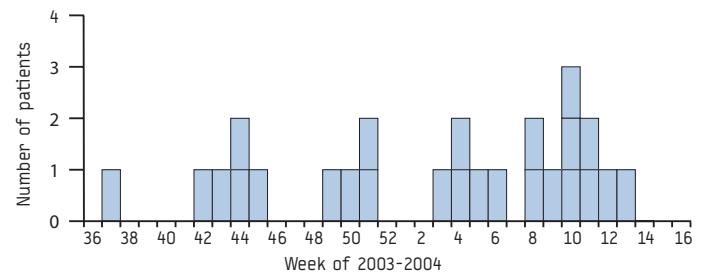
Distribution of VTEC O157:H- infections in Denmark, September 2003-March 2004



Supermarket chain A branches by municipality or county VTEC O157:H- incidence by municipality or county*
 * There were 13 patients in the municipality of Copenhagen (shown within the circle). In counties where cases occurred, there were between one and four patients.

FIGURE 2

Number of VTEC O157:H- cases with identical PFGE pattern per week of onset, n=25. Denmark, September 2003 to March 2004



Initial interviews and a descriptive epidemiological assessment showed that the infections were acquired domestically and that the patients shared no common exposures such as visits to restaurants, shops, recreational facilities or farms. Of 12 patients, 8 had bought food at supermarket chain A and 11 at chain B, although from different shops.

Case-control study

Eleven cases and 55 controls were included in the study. Ten patients reported buying food at supermarket A, matched odds ratio (OR): 8.7 (95% confidence intervals (CI): 1.1-71) and nine at supermarket B, OR: 0.99 (95% CI: 0.2-5.5). Organic milk from dairy X was the only food item found to be consistently associated with an increased risk. In an initial analysis including all case-control sets, the OR was 3.6 (95% CI: 0.9-14). However, three of the 11 cases were possible secondary cases (from kindergartens) and when these were excluded, five out of eight cases reported drinking milk from dairy X bought in supermarket A, in comparison with five of 39 control persons, OR: 8.7 (95% CI: 1.6-48). Initial hypotheses concerning beef sausage, other kinds of sausage, beef, green pepper and grapes could not be verified in the analysis. Consumption of beef sausage from a particular company was associated with an increased risk, OR: 8.5 (95% CI: 0.8-90), but only three patients had consumed sausage from this company, and they had each eaten a different type of sausage. Among seven cases and 14 controls who reported shopping exclusively at supermarket A, milk from dairy X was the only product that tended to be associated with an increased risk of illness OR: 6.6 (95% CI: 0.7-61). After completion of the case-control study, all patients were

interviewed about milk consumption and 13 of 22 patients reported drinking milk from dairy X. All of the patients during the outbreak period lived in municipalities served by supermarket chain A, which only operates in and around Copenhagen [FIGURE 1].

Environmental investigations and control measures

Dairy X is an independent dairy that produces approximately 80 000 litres of organic milk each week, and also produces a range of other organic dairy products. Based on the suspicion that milk from dairy X was a likely vehicle, the milk was recalled and dairy X temporarily closed on 25 March 2004. A careful inspection at the premises did not reveal any major deficiencies. According to the dairy's records, all alkaline phosphatase tests were negative. Finally, neither environmental investigations nor analyses of 42 samples of raw milk revealed any VTEC O157 strains.

Discussion

The case-control analysis clearly indicated that buying foodstuffs in supermarket A was associated with an increased risk of illness due to VTEC O157:H- infection, whereas other supermarkets and shops were not. Organic milk from dairy X was the only food item found to be associated with an increased risk.

Dairy X produces organic milk that is distributed to several parts of Denmark. One of the most popular products, however, is milk from Jersey cows, which was only distributed through supermarket A. Jersey milk is characterised by higher viscosity, protein and fat content than milk from red and black/white dairy cattle. The production of organic Jersey milk differs from the normal procedures for pasteurisation of milk, because the whole raw milk is pasteurised prior to fractioning. The Jersey milk from dairy X originated exclusively from about 15 herds of Jersey cows. From 1 September 2003 until 30 March 2004 a total of 1.25 million litre cartons of Jersey milk were sold from supermarket A, and 41% of these were sold in the Copenhagen municipality (central Copenhagen), where 52% of registered cases lived, equivalent to an attack-rate of 2 cases per 100 000 litres of milk. We therefore assume that the outbreak was caused by low-degree contaminated Jersey milk from dairy X distributed in a specific supermarket chain in Copenhagen and the municipalities north of Copenhagen.

The outbreak strain possessed the *vtx1*, *vtx2c*, and the *eae* genes. Though the PFGE profile of the outbreak-strain was rare, the specific combination of the *vtx1* and *vtx2c* genes is common in *E. coli* O157 strains isolated from Danish cattle. Furthermore, phage type 8 is among the four most common phage types in bovine VTEC O157 isolates obtained in Denmark during the period 1994-2001 [17]. The presence of the variant *vtx2c* rather than the variant *vtx2* could be the reason for the relatively mild symptoms experienced by most patients in this outbreak [22,23]. It has been suggested that *vtx2c* strains produce lower amounts of verocytotoxin than *vtx2* strains [22].

Investigations at the dairy did not reveal faults in the production process that could explain the outbreak, and the results of alkaline phosphatase test (which demonstrates whether the mammalian phosphatase enzyme present in raw milk is inactivated by pasteurisation) were negative. No VTEC strains were recovered from pooled raw milk samples or from environmental samples taken at the dairy. However, sampling from individual cows in the herds, which was not carried out, might have yielded useful results. For example, in an outbreak of VTEC O157 phage type 21/28 associated with pasteurised milk, no positive samples were obtained from the milk at the time of the outbreak. However, several calf pen samples and samples from slurry were positive; these strains were indistinguishable from the outbreak strain [11]. In the outbreak reported here, it was decided that such extensive examinations were not necessary, and so microbiological confirmation of the source of the outbreak was not obtained. It is likely that the present outbreak was due to a limited environmental contamination of the Jersey milk after pasteurisation, or to intermittent inadequate heat treatment due to the specific properties of the milk, but these possible hypotheses remain speculative. The outbreak ended when interventions were carried out at the dairy.

The outbreak was detected through the national laboratory surveillance, thanks to the ongoing molecular typing of isolates. Descriptive and analytical epidemiological evidence indicated that the outbreak was caused by Jersey milk from a specific organic dairy, which was contaminated with VTEC O157 at a very low level and distributed through a specific supermarket chain, although this was not confirmed microbiologically. If feasible, we recommend that in future outbreaks, bacteriological examinations be extended to include dairy cattle, to improve the likelihood of microbiological confirmation of suspected milkborne outbreaks. The specific pasteurisation procedures of Jersey milk, or post-pasteurisation contamination, may have been critical factors for the outbreak.

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ORIGINAL ARTICLES

Outbreak report

AN OUTBREAK OF AIRBORNE TULARAEMIA IN FRANCE, AUGUST 2004

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Fifteen tularaemia cases were identified after a holiday spent at a converted mill in the Vendée region in France, between 9 and 12 August 2004.

The mill was visited, and descriptive, retrospective cohort and environmental investigations were conducted. The 39 people who had stayed at the mill between 24 July and 11 August were asked about symptoms, exposure to food and animals, and leisure activities.

A case was defined as a person with evidence of fever and a positive serology (seroconversion or significant rise in antibody titre, or a single titre) ≥ 40 . Culture for *Francisella tularensis* and polymerase chain reaction (PCR) diagnosis was carried out for drinking water, firewood, and domestic animals at the mill.

Fifteen cases of tularaemia (38%) were confirmed. Twelve of the cases (80%) had the pulmonary form. None of the patients was admitted to hospital.

There was a strong association between infection and participation in a dinner at the mill on 4 August ($p < 10^{-8}$). One of the three dogs present in the dining room was serologically positive for *F. tularensis*.

Results of analysis of environmental samples were negative.

These investigations confirmed the occurrence of a cluster of 15 tularaemia cases, in patients who were infected on the evening of 4 August, in a mill in Vendée, an endemic area for tularaemia. The investigations highlight the existence of nonspecific and benign pulmonary forms of the illness in France.

The pulmonary form of infection in the human cases and the positive serology of the dog suggest contamination by inhalation of contaminated particles from the dog's fur disseminated by the dog shaking itself.

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Introduction

Tularaemia is a bacterial zoonosis caused by *Francisella tularensis* [1]. Humans may become infected through bites from infected ticks or other insects, contact with infected animals or contaminated animal products, consumption of vegetables, water or earth contaminated by the faeces or corpses of infected animals, or inhalation of aerosolised bacteria. The median incubation period for the disease is 3 to 5 days (range: 1 to 14 days) [2]. The clinical form of tularaemia depends on the route of entry of the bacterium into the body: ulceroglandular tularaemia is involved if transmission is transcutaneous; pulmonary and typhoidal tularaemia if caused by inhalation; oropharyngeal tularaemia if by ingestion.

On 21 August 2004 a general practitioner informed the local Direction Départementale des Affaires Sanitaires et Sociales (Departmental Health and Social Services Division, Ddass) of 15 cases of flu-like infections in patients who had spent 4 August 2004 at a mill that had been converted into a home in Vendée, western France. On 8 September, blood tests confirmed the diagnosis of tularaemia for 3 of the 15 patients.

Because of the similarity of symptoms in the other 12 patients who had been at the same place on the same date, a diagnosis of tularaemia, and a common source of contamination were suspected for the whole group. Epidemiological and environmental investigations were performed to confirm the diagnosis and identify the source of contamination and the mode(s) of transmission with a view to taking appropriate outbreak control measures.

Methods

Epidemiological investigation

This investigation included a visit to the mill, a descriptive investigation of the cases and a retrospective cohort study of all the subjects who stayed at the mill from 24 July to 11 August 2004.

A site visit was conducted to describe the house and its surroundings, and to interview the owners in order to establish a list of animals and humans who were present during the period under study and retrace their activities during that time, particularly on 4 August.

A case was defined as any patient with fever and a positive blood test (agglutination): either a seroconversion, or a significant increase in antibody titres, or a single titer greater than or equal to 40. The blood tests for all the patients were performed by the national reference centre for tularaemia.

The clinical forms were classified as pulmonary tularaemia (minimum of one respiratory symptom or abnormalities on the chest x ray picture) and typhoidal tularaemia based on clinical and biological information collected from the physician.