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Verotoxin producing *Escherichia coli* O 157 infections associated with the consumption of yoghurt

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SUMMARY

Sixteen cases of verotoxin producing *Escherichia coli* (VTEC) O 157 : H7 Phage Type 49 infection were identified in the North West of England from 1 September to 1 November 1991, eight of whom lived in or around the same large town. Eleven of the cases were aged 10 years or less, and five of the affected children developed haemolytic uraemic syndrome. A case control study demonstrated a strong association between VTEC O 157 : H7 PT 49 infection and the consumption of a locally produced live yoghurt. This is the first time that an outbreak of VTEC O 157 infection has been linked to the consumption of yoghurt and this vehicle of infection should be considered when investigating such outbreaks in future.

INTRODUCTION

Verotoxin producing *Escherichia coli* (VTEC) O 157 was first recognized as a human pathogen in 1982 (1). Infection by the organism produces a wide spectrum of clinical disease from asymptomatic infection, through mild diarrhoea to more severe manifestations including haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). HUS is characterized by acute renal failure, microangiopathic haemolytic anaemia and thrombocytopenia. While all age groups may be affected, it is more common in infants and young children.

The outbreak

In late Autumn of 1991, an outbreak of VTEC infection came to light when nine children in North Western Region were shown to have *E. coli* O 157 : H7 infections. Subsequently, the isolates were identified as phage type (PT) 49 by Public Health

Laboratory Service (PHLS)-Division of Enteric Pathogens (DEP). Preliminary investigations suggested that, prior to the onset of illness, 8 of the 9 children had eaten the same brand of yoghurt produced by a local dairy. The cases had eaten a variety of different flavours of the yoghurt.

The dairy

The yoghurt was a live, flavoured yoghurt produced from pasteurized full fat milk and marketed specifically for children. It was usually made once a week in a batch of 200 dozen pots depending on demand. The manufacturers also made other live yoghurts, producing 1200–1300 dozen pots of skimmed milk yoghurt a day and 220 dozen pots of whole milk yoghurt twice a week. The live culture and the same make of fruit puree was used for all types of yoghurt (except for the chocolate flavour which was flavoured with cocoa powder). The farm only used milk from its own cattle.

METHODS

Epidemiological

Active case finding and examination of both local and national data confirmed that cases were localized to the North Western Region.

Case definition: a case was defined as a patient living in the North Western Region with confirmed VTEC O 157:H7 PT 49 infection and a diarrhoeal illness or HUS since 1 September 1991.

Patients were excluded if they had a history of travel outside the UK in the 7 days before the onset of symptoms.

A case control study was undertaken to test the hypothesis that infection with VTEC O 157:H7 PT 49 was associated with the consumption of the locally produced yoghurt.

Case control study

Selection of controls

Each case was asked to nominate three controls. These were one household control and two neighbourhood controls. The household control was the member of the household nearest in age to the case and had not been ill with a gastrointestinal illness in the 10 days before or after the onset of illness in the case. If there was no household control within 5 years of age of the case, then the parent or case was asked to nominate three neighbourhood controls. For each case, the neighbourhood controls were matched for age, and sex if aged over 10 years.

Questionnaires

The case control study was conducted using a questionnaire which identified name, age, sex, address, clinical features, household contacts with diarrhoea, a history of foodstuffs eaten in the week before illness and recent foreign travel.

Questionnaires administered to the cases and controls were similar, and the questionnaires for the controls asked about food history/preferences during the same time period as the relevant case.

The questionnaires were administered by telephone interview. If the case or control was not available by telephone, a face to face interview took place.

The questionnaires were coded entered and verified on computer at CDSC. Data were analysed using the Epi-Info statistical package and tests of significance using exact methods (2).

Microbiology and environmental

The local PHL which reported most of the cases, routinely looks for *E. coli* O 157 on all specimens of bloody diarrhoea and examined all fluid faecal samples once the outbreak had been identified. All *E. coli* O 157 isolates identified were sent to PHLS-DEP for confirmation and further typing.

The dairy was visited and several areas where potential cross contamination could occur were identified and the owner was advised accordingly.

Following the results of the case control study, staff from the local Veterinary Investigation Centre (MAFF) and PHL visited the farm and took rectal swabs from the cattle and samples of fresh cow faeces.

RESULTS

Epidemiology

Descriptive

In the period 1 September to 1 November 1991, 22 patients with *E. coli* O 157 infections were identified in the North Western Region. Sixteen fulfilled the case definition by yielding *E. coli* which were both verotoxin producing and PT 49. Six patients, were identified as having *E. coli* O 157 infections, but were not included in the study as they were subsequently found to be phage types other than PT 49. The onset dates of the 16 cases of infection are shown in Figure 1 and suggest a main cluster of 12 cases with onset dates between the 6 and 19 October. Of the four cases of infection occurring outside the main cluster, three lived in a town some miles from the other cases and had onset of illness dates in mid-September.

The age range of the cases was 1–56 years. Eleven of the 16 cases, and 10 out of the cluster of 12 cases were aged 10 years or less.

Five cases lived in one area of a large town and 3 cases lived in other areas of the same town (all children with onset dates within the main cluster); 4 lived in another town approximately 30 miles from the first town and 4 cases came from different towns in the North West of England.

Of the 16 cases, 13 had bloody diarrhoea and 3 reported diarrhoea. Thirteen (82%) were admitted to hospital and their length of stay ranged from 1 day to over 3 weeks. Five children had HUS.

Analytical study

All 16 cases and 42 controls were interviewed by telephone. Three controls were interviewed for each of 11 cases, 2 controls were interviewed for each of 4 cases and 1 case could only give 1 control.

Preliminary analysis. A range of possible risk factors was examined using unmatched single variable analysis. The only statistically significant association found was that between illness and the consumption of the implicated yoghurt with an odds ratio = 10 (Table 1).

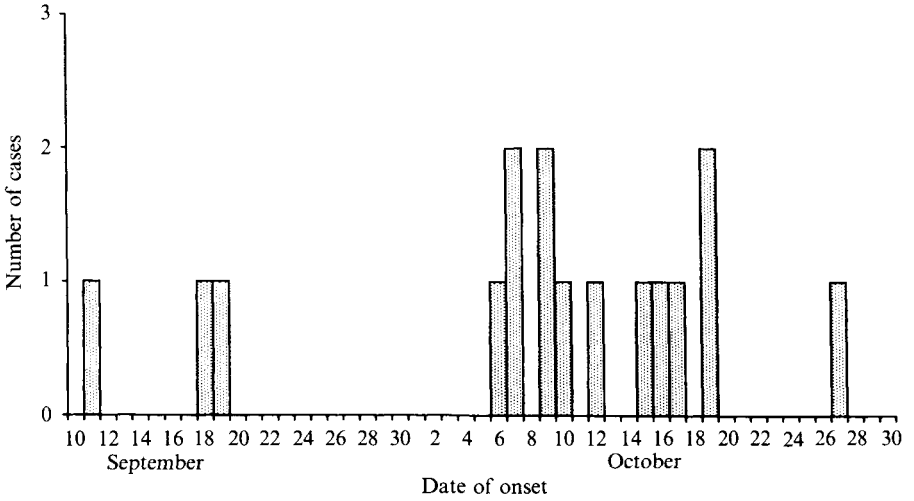

Figure 1. *E. coli* O 157 phage Type 49. Dates of onset of symptoms of cases.

Matched analysis. Due to the small numbers involved, exact methods [2] were used to assess the chance (p value) of this pattern of exposure occurring in matched sets of cases and controls assuming the null hypothesis that exposure (eating the locally produced yoghurt) has no effect. Selecting the household controls only, this result did not reach statistical significance, probably because the numbers were too small to detect an association (only seven matched sets were obtained). Selecting neighbourhood controls, there was significant evidence ($P = 0{\cdot}00033$) to suggest that consumption of the yoghurt was associated with illness.

Eight cases, who were in the cluster of 12 cases, definitely ate the yoghurt in the week before onset of illness and 1 case ate the yoghurt some time in the 2 weeks prior to the onset of his illness. Four out of 39 controls had eaten the yoghurt in the week prior to the onset of illness in their matched case (3 controls were not sure if they had eaten the yoghurt).

Microbiology and environmental

Examination of all fluid faecal specimens at the local PHL once the outbreak was identified revealed no other cases.

Yoghurts (produced after the suspect batch) were tested but *E. coli* O 157 was not isolated.

Examination of the production processes at the farm identified two systems. Raw whole milk and skimmed milk were stored in individual tanks which were emptied and disinfected daily. The majority of yoghurts produced by the farm were from skimmed milk which was transferred to the pasteuriser by a fixed pipe system (and cleaned in place). Raw whole milk was transferred to a pasteurizer using a mobile pump which was then used, without intermediate disinfection, to distribute the pasteurized milk inoculated with starter culture into churns. No more than one batch of whole milk yoghurts was produced on any day.

Others areas where theoretical cross contamination of the yoghurt could have occurred were identified. These were that it was possible to walk into the yoghurt production plant with muddied boots (because access to that area was directly off

Table 1. *Unmatched single variable analysis of food preferences of cases and controls*

	Case		Control		Odds ratio (95% confidence interval)
	Ate	Did not eat	Ate	Did not eat	
'Fast food' hamburgers	2	12	3	33	1·8 (0·1, 17·9)
Chicken	0	13	5	32	—
Sausages	7	7	20	14	0·7 (0·2, 3·0)
Sausage meat	2	13	6	30	0·8 (0·1, 5·0)
Cold meats	8	7	27	10	0·4 (0·1, 1.8)
Hamburger/beefburger	2	14	11	22	0·3 (0·0, 1.7)
Unpasteurized milk	0	16	1	39	—
Yoghurts:					
Ski	0	16	1	36	—
Local live yoghurt	8	7	4	35	10·0** (1·9, 56·1)
Munch Bunch	2	14	4	34	1·2 (0·1, 9·6)
Fiendish Feet	1	15	2	36	1·2 (0·0, 24·6)
Thick & Creamy	1	14	0	38	—

the farm yard) and that churns of yoghurt were placed directly on the floor of the production area and up-ended several times during the stages of the yoghurt-making process. It was considered by inspectors that water or debris on the bases of the churns could drop into the cartoning reservoir. In addition, there was no record kept of the pasteurizing temperature and time. Recommendations were made to the owners of the dairy to modify the production process to overcome the problems outlined above. It was noted that once the yoghurts had been produced, they were stored at a temperature below 8 °C and delivered to the outlets in refrigerated vans.

E. coli O 157 was not isolated from samples of raw or pasteurized whole and skimmed milks or the implicated brand of yoghurt collected during visits to the farm.

Following the epidemiological study, 180 rectal swabs from the cattle on the farm were examined by PHLS-DEP using DNA probes for VT1 and VT2, and VTEC were found in 31 samples (17%) but none were *E. coli* O 157 [3].

DISCUSSION

There were 16 cases of VTEC O 157 PT 49 infections in the North Western Region from 1 September to 1 November 1991. Twelve cases of infection occurred in a cluster with onset dates from the 6–19 October. Most of the cases were children.

A case control study confirmed local investigations which suggested the VTEC O 157 PT 49 outbreak was associated with the consumption of a particular brand of yoghurt marketed for children. Out of the cluster of 12 cases, 8 definitely ate the yoghurt in the week before onset of illness and one case ate the yoghurt sometime in the 2 weeks before the onset of illness. Although one case had never consumed the yoghurt, her brother regularly ate them at school (his faecal sample was later shown to be negative). Another case who had not eaten the yoghurt, had returned

from the Canary Islands 8 days before the onset of her symptoms. Although the accepted incubation period of *E. coli* O 157 infections is 3–4 days [4], several studies have described longer incubations of 12–14 days for definite cases [5, 6]. In future studies, it is suggested that enquiries be made about food consumption in the 2-week period prior to the onset of symptoms.

Also, phage type 49 is one of the commonest phage types of *E. coli* O 157 identified in England and Wales, and so a number of cases might be expected to occur over this period.

Although beef products have mostly been identified as the food vehicle for outbreaks of VTEC O 157 infections [6, 7], there have also been incidents of VTEC infections following the consumption of unpasteurised milk [5, 8]. This is the first reported outbreak in which yoghurt has been involved.

The suspect batch contained about 200 dozen pots and although the product was subsequently removed from sale by the retailer, many yoghurts were purchased and presumably consumed. Despite this and a programme of active case finding, we discovered relatively few people with *E. coli* infection. Low attack rates have been found in other outbreaks and have been attributed to variation in host susceptibility and the wide range of severity of clinical illness [9]. In outbreak situations, studies have estimated that the proportion of persons presenting with bloody diarrhoea varies between 14–75% [10]. Since 13 out of 16 cases in this study reported bloody diarrhoea, there is the possibility that despite efforts to identify cases, a number of those exposed developed milder forms of the disease and remained undetected. In this incident, it is also possible that although no specific flavour was involved, only part of the batch was contaminated. However, it needs to be borne in mind that the numbers of *E. coli* surviving in yoghurt decline during storage [11], thus yoghurt consumed several days after purchase may be less likely to be followed by illness.

In this outbreak, 5 of 12 people in the main cluster of cases (5 of all 16 cases identified) developed HUS, whereas previous studies estimate that about 2–7% of those with *E. coli* O 157 : H7 infections develop HUS (9). A combination of factors may account for the apparently high proportion of cases with this complication. These include that the yoghurt was specifically marketed for infants and young children in whom the development of HUS is more common, and as discussed above, we only detected the more severe cases. Alternatively, the organism involved may have been particularly virulent.

The childrens' yoghurt was produced once a week in one batch unrelated to other yoghurt preparation and no cases of *E. coli* O 157 infection were detected in persons who had consumed other batches of flavoured or unflavoured yoghurt produced by the dairy.

Although a number of potential problems at the dairy were identified, the general standard of hygiene was high. The milk might have been inadequately pasteurized, but it is more likely that the milk became contaminated after pasteurization, either due to inadequate cleaning of the mobile pump or by farm yard matter.

This is the first time that an outbreak of *E. coli* O 157 infection has been linked to the consumption of yoghurt. This vehicle of infection should be considered when investigating such outbreaks in future.

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REFERENCES

1. Riley LW, Remis RS, Helgerson SD. et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med 1983; **308**: 681–5.
2. Tillet HE. Statistical analysis of case-control studies of communicable diseases. Int J Epidemiol 1986; **15**: 126–33.
3. Willshaw GA, Cheasty T, Jiggle B, Rowe B, Gibbons D, Hutchinson DN. Vero cytotoxin-producing *Escherichia coli* in a herd of dairy cattle. Vet Rec 1993; **132**: 96.
4. Riley LW. The epidemiologic, clinical and microbiologic features of hemorrhagic colitis. Ann Rev Microbiol 1987; **41**: 383–407.
5. Duncan L, Mai V, Carter A, Carlson JAK, Borczyk A, Karmali MA. Outbreak of gastrointestinal disease-Ontario. Can Dis Weekly Rep 1987; **13–2**: 5–8.
6. Ryan CA, Tauxe RV, Hosek GW, et al. *Escherichia coli* O 157:H7 diarrhoea in a nursing home: clinical, epidemiological, and pathological findings. J Infect Dis 1986; **154**: 631–8.
7. Belongia EA, MacDonald KL, Parham GL, et al. An outbreak of *Escherichia coli* O 157:H7 associated with consumption of precooked meat patties. J Infect Dis 1991; **164**: 338–43.
8. Borczyk AA, Karmali MA, Lior H, Duncan LMC. Bovine reservoir for verotoxin-producing *Escherichia coli* O 157:H7. Lancet 1987; i: 98.
9. Griffin PM, Ostroff SM, Tauxe RV, et al. Illnesses associated with *Escherichia coli* O 157:H7 infections. Ann Intern Med 1988; **109**: 705–12.
10. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O 157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev 1991; **13**: 60–98.
11. Goel MC, Kulshrestha DC, Marth EH, Francis DN, Bradshaw JG, Read RB. Fate of coliforms in yoghurt, buttermilk, sour cream, and cottage cheese during refrigerated storage. J Milk Food Technol 1971; **34**: 54–8.