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Multiple norovirus outbreaks among workplace canteen users in Finland, July 2006

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SUMMARY

Multiple gastroenteritis outbreaks occurred between 25 and 31 July 2006 in 10 workplace canteens in south-western Finland. One vegetable processing plant provided raw vegetables to all the canteens. We conducted cohort studies in the three most visited canteens and environmental investigations in the kitchens and the plant. Patients' stools, food, water and environmental samples were tested for enteric bacteria and viruses. Of the three canteens, 150/273 respondents (response rate 82%) had gastroenteritis. Consumption of mixed raw vegetables was significantly associated with the illness but no single vegetable explains the outbreak. An identical norovirus GII.1 genotype was detected from all genotyped patient samples. Water, food, and environmental samples were negative for norovirus. The facilities had appropriate hygienic conditions and no staff member had gastroenteritis prior to the outbreak. Tracing back the vegetables to the farm level proved unsuccessful. This was the largest foodborne norovirus outbreak in Finland.

INTRODUCTION

Noroviruses are the most common aetiological agents of viral gastroenteritis outbreaks in Europe and the United States [1–3]. They have a low infectious dose, and can be transmitted by various routes including food, water, and environmental contamination, as well as person-to-person contact. After a short incubation period of 12–48 h, the infection typically results in a mild self-limiting gastroenteritis that lasts from 12–60 h [4]. Asymptomatic infections are common. Annually 100–800 microbiologically confirmed norovirus cases are reported in Finland [5], some of which are associated with food- or waterborne outbreaks.

On 28 July 2006, the National Public Health Institute was notified of several gastroenteritis outbreaks in the Pirkanmaa region, south-western Finland. The outbreaks occurred among people who had taken their meals in different workplace canteens in the city of Tampere and neighbouring municipalities. Although the canteens were catered by different restaurants, the raw vegetables and salads they served originated from one vegetable processing plant. We conducted epidemiological, laboratory, and environmental investigations to determine the extent and source of the outbreak.

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MATERIALS AND METHODS

Epidemiological investigation

The occupational health services of the affected workplaces, together with local environmental health units, collected information on the number of canteen users who had acute gastroenteritis between 25 and 31 July. Based on the results of the initial case finding, we decided to focus further investigations on the three most visited canteens: canteen A in the municipality of Valkeakoski and canteens B and C in the city of Tampere. In the analytical study, we attempted to exclude probable secondary cases by defining a case more specifically as a person who used canteens A, B or C between 25 and 26 July, and had an onset of diarrhoea or vomiting between 26 and 27 July.

A standardized questionnaire was distributed to all employees who regularly used canteens A, B or C. Demographic and clinical data, as well as data on the food items and beverages consumed, were collected by self-administered questionnaires within 1 week following notification of the outbreak.

We conducted retrospective cohort analyses in the three canteens separately, referred to as cohorts A, B and C. Using univariate analysis we calculated the relative risks (RR) with 95% confidence intervals (CI) of the consumed food items, using Fisher's exact test to check probability. Multivariate analyses were carried out using logistic regression, because the model fits well in a wide range of data and the given odds ratios are a good summary of the association even in a cohort study [6]. Taking into account that the only epidemiological link was raw vegetables and salads as possible vehicle(s) of transmission, the multivariate analysis was carried out with these food items.

Based on the daily information on vegetable consumption we created new variables (combined vegetables) which indicated whether the persons were exposed to any of the suspected vegetables in the canteen on 25 or 26 July. For example, among users of canteen A those who ate tomato–leek salad and/or cucumber slices on 25 July were exposed to combined vegetables on 25 July. By using these variables we repeated the univariate and multivariate analysis.

Laboratory investigation

Stool samples from patients with gastroenteritis were sent for enteric bacterial and viral analyses: three, five, and three samples from cohorts A, B and C, respectively. The samples were investigated for noroviruses by quantitative reverse transcriptase PCR test at the Department of Virology, Helsinki University Central Hospital Diagnostics Laboratory [7]. The genotype of five noroviruses (1–2 per cohort) was determined by sequence analysis according to Vinje *et al.* [8] at the University of Helsinki. Amplicons of 253 bp in the capsid region and 300 bp in the polymerase region were amplified using One-step RT–PCR kit (Qiagen, Hilden, Germany) for nucleic acid sequence determination. Amplicons were sent to the Institute of Biotechnology, University of Helsinki, for sequence determination. Phylogenetic analysis of the nucleotide sequences was performed with the ClustalW program. The nucleotide sequences have been deposited at Genbank (accession nos. EU029107-11).

Four 1-litre samples of tap water taken from the canteens were analysed for noroviruses with the adsorption-elution method [9].

A selection of food samples, mainly vegetables from each canteen, was analysed for noroviruses. Food samples were investigated for viruses by eluting them with glycine buffer (pH 8·5) combined with microfiltration [10]. Rinsing the lettuce with a larger volume of PBS and filtration through a positively charged membrane was also performed. In addition, vegetables processed in week 30 (24–28 July), and stored frozen at the vegetable processing plant were obtained for viral analysis. Five hygiene swabs taken from cutting machines and edges and table surfaces from the vegetable processing plant were investigated for noroviruses.

Environmental investigation

Environmental investigations were carried out between 28 and 31 July in the kitchens of the three canteens. Moreover, site inspections were conducted at three other workplace canteens, where cases of acute gastroenteritis were reported. These inspections focused on food processing, hygiene conditions, efficacy of in-house control, and the related health status of kitchen staff.

The local environmental health inspectors visited the premises of the vegetable processing plant on 31 July. They examined the hygiene conditions, fresh produce processing, trace-back register of processed food items, reported diseases among the staff, and in-house control.

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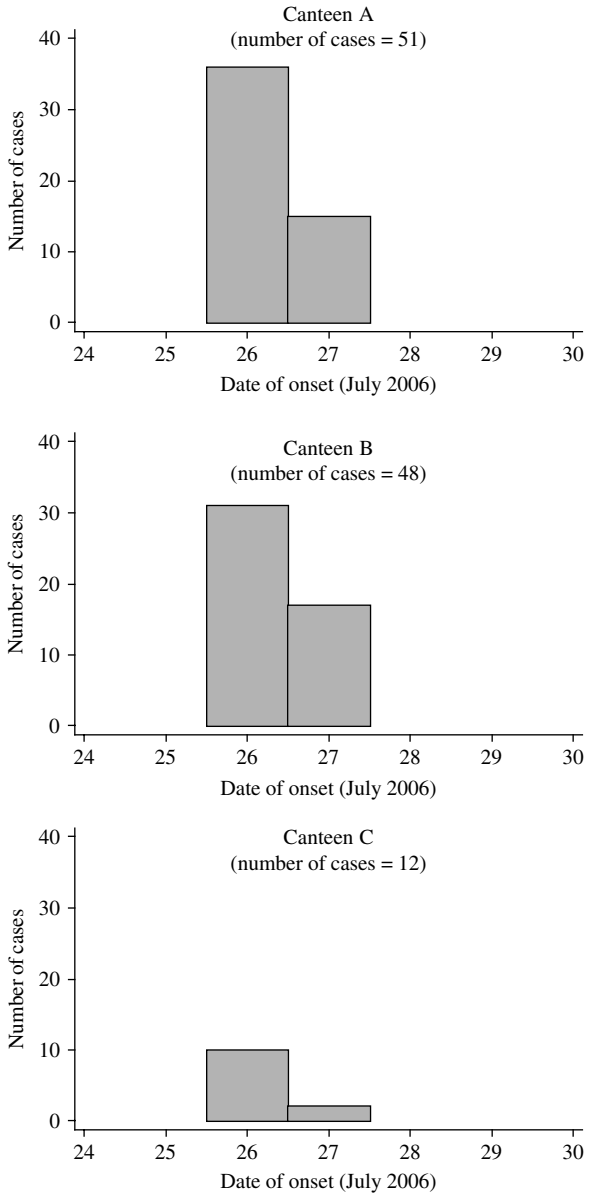

Fig. 1. Onset of symptoms among cases during norovirus outbreaks in Pirkanmaa region, Finland, July 2006 in canteens A, B and C.

RESULTS

Descriptive and analytical findings

Altogether ten workplace canteens were affected by the outbreak, resulting in over 400 cases of acute gastroenteritis between 25 and 31 July. In the three most visited canteens, there were 150 patients with gastroenteritis among the 273 responding canteen users (response rate 82%, attack rate 55%), and 111 of them met the case definition (Fig. 1).

In cohorts A and C, the proportion of males was greater than 80%. The median age was 37 years (range 16–62 years) and 38 years (range 19–64), respectively. Age and sex were not reported for cohort B. The reported incubation period ranged from 4 h to 54 h. Overall in the three cohorts, the most common clinical symptoms were vomiting (89%), diarrhoea (80%), and abdominal pain (80%). The mean duration of the illness was 49·2 h (range 5–156 h). Almost 70 patients sought medical attention but none were hospitalized.

Results of the analytical studies showed that in canteens A and B, apart from the raw vegetables and salads, no other food item was significantly associated with the illness. In other findings the cohorts differed from each other as follows.

Canteen A had 123 respondents who used the canteen on 25 or 26 July, of which 51 met the case definition. Univariate analysis suggested that 3/8 raw vegetables/salads were significantly associated with illness and one was associated by borderline probability (Table 1). The multivariate analysis did not show a significant association of any raw vegetable with the illness. Analysis of the daily combined vegetables showed that vegetables served on 25 and 26 July were associated by borderline probability with the illness. As almost everyone ate the same food, further differentiation was not possible between them with the applied statistical methods due to the multicollinearity of variables. Exposure to daily combined vegetables on 25 and/or 26 July altogether was significantly associated with the illness (overall P value of the model = 0·012).

Canteen B had 100 respondents who used the canteen on 25 or 26 July, of whom 48 were cases. Multivariate analysis showed that four out of five suspected vegetables were significantly associated with the illness (Table 2). The multivariate analysis of daily combined vegetables confirmed that the vegetables served on 25 and 26 July were significantly associated with the illness (overall P value of the model $<$ 0·0001).

Canteen C had 24 respondents who used the canteen on 25 or 26 July, of which 12 were cases. No food item served in canteen C was significantly associated with illness either by univariate or multivariate analysis.

Laboratory investigations

Bacterial cultures of the stool samples from patients with gastroenteritis and from kitchen staff yielded no enteric pathogen.

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Table 1. *Univariate relative risks (RR) and multivariate odds ratios (OR) for cases among canteen A users by type of food consumed during the norovirus outbreaks in Pirkanmaa region, Finland, July 2006*

Food consumed	Attack rate among exposed	Univariate		Multivariate	
		RR	*P*	OR	*P*
Tomato–leek salad (25 July)	50·9	2·0 (1·0–4·5)	0·048	1·1 (0·9–1·4)	0·344
Cucumber slices (25 July)	50·7	2·0 (0·9–5·2)	0·095	1·3 (0·8–2·0)	0·256
Chinese cabbage and lettuce strip mix (26 July)	53·4	2·7 (1·1–7·0)	0·011	0·5 (0·3–1·2)	0·116
Paprika strips (26 July)	57·8	2·0 (1·1–3·4)	0·013	1·3 (0·7–2·3)	0·463
Salads on 25 July	50·6	1·7 (1·0–2·9)	0·036	2·1 (0·9–5·0)	0·085
Salads on 26 July	51·3	1·8 (1·1–3·0)	0·023	2·3 (1·0–5·4)	0·055

Table 2. *Univariate relative risks (RR) and multivariate odds ratios (OR) for cases among canteen B users by type of food consumed during the norovirus outbreaks in Pirkanmaa region, Finland, July 2006*

Food consumed	Attack rate among exposed	Univariate		Multivariate	
		RR	*P*	OR	*P*
Braised vegetables (25 July)	61·2	1·8 (1·1–3·0)	0·026	4·6 (1·2–17·4)	0·022
Cabbage salad (25 July)	56·1	2·0 (0·9–4·4)	0·061	0·9 (0·2–4·1)	0·889
Swede–black radish strip mix (26 July)	61·8	1·8 (1·1–2·9)	0·012	2·8 (1·0–8·4)	0·061
Rice–egg salad (26 July)	75·0	1·7 (1·1–2·4)	0·052	4·7 (1·0–21·1)	0·046
Braised broccoli (26 July)	70·8	1·6 (1·1–2·4)	0·032	5·3 (1·1–25·4)	0·035
Salads on 25 July	54·4	1·6 (0·9–2·7)	0·086	2·7 (1·1–7·2)	0·039
Salads on 26 July	63·1	3·2 (1·6–6·3)	<0·001	7·5 (2·8–20·5)	<0·001

All 11 stool samples were positive for genogroup II noroviruses by real-time RT–PCR test. All sequenced samples were of genotype GII.1 and were 100% identical with each other, both in the partial polymerase and capsid region (Fig. 2). Our nucleic acid sequences had, however, only 73·6% identity with that of the GII.1 prototype strain Hawaii in the capsid region. The most closely related sequence in Genbank was the Japanese Gifu/96 strain (accession no. AB045603) with sequence identity of 94·9%. In the polymerase region, nucleotide sequences showed an identity of 84·8% and 95·7% with the strains Hawaii and Wortley, respectively.

All the water, food, and environmental samples were negative for noroviruses.

Environmental investigations

The kitchens of the six canteens inspected were found to be in good hygienic condition. No gastrointestinal illness was reported among the staff members prior to the outbreak. In canteen C, three members of the kitchen staff had acute gastroenteritis after 26 July. The in-house control worked properly, and no shortcomings were found in food processing.

Good hygiene conditions were also found at the vegetable food processing plant. No employee of the plant reported disease with gastrointestinal symptoms in the 2 weeks prior to the outbreak. The root vegetables were handled on a separate production line from other fresh produce and cold water was used to wash all the vegetables. The processing lines were washed with cold water between batches of fresh produce. The plant purchased the vegetables from several suppliers: directly from farmers, from wholesalers, as well as from a bulk supplier abroad. It was extremely difficult to trace back the origin of the raw vegetables used in the facility, because they cut and grated different kinds of vegetables from various sources at the same time, and usually produced mixed salads. The real origin of the vegetables distributed during 25–31 July remains unknown.

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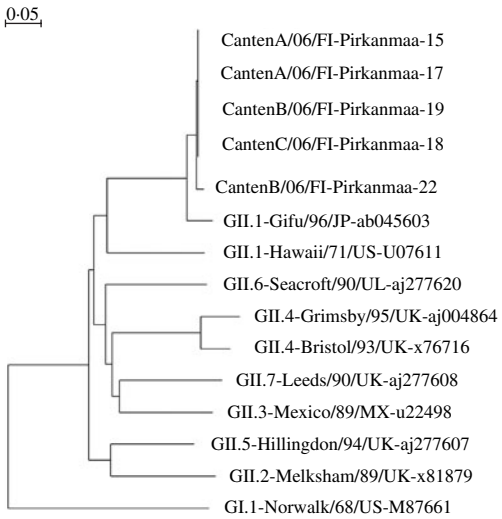

Fig. 2. Phylogenetic tree derived from 14 norovirus genogroup II nucleotide sequences and one genogroup I sequence from the capsid region. Our sequences were aligned together with the following EMBL/Genbank norovirus genotype strains Hawaii/71/US (U07611), Melksham/89/UK (X81879), Mexico/89/MX (U22498), Grimsby/95/UK (AJ004864), Bristol/93/UK (X76716), Hillingdon/94/UK (AJ277607), Seacroft/90/UK (AJ277620), Leeds/90/UK (AJ277608), Gifu/96/JP (AB045603), and Norwalk/68/US (M87661). The bar shows genetic distance of 0·05. Branch lengths are related to degree of divergence between sequences.

DISCUSSION

Multiple foodborne gastroenteritis outbreaks occurred in several workplace canteens in Pirkanmaa region, Finland, during the last week of July 2006. The attack rate among canteen users from the three investigated canteens was high; more than half of the responding canteen users had gastroenteritis during the outbreak. Norovirus was the only pathogen detected from patient samples.

Sequence analysis revealed that identical noroviruses infected people eating in different canteens suggesting a common source of infection. The genotype GII.1 noroviruses have not been detected in recent Finnish outbreaks. In 2000–2001 they were circulating commonly, but then the sequences were nearly identical to Hawaii strain [9], unlike the sequences reported in the present study. Later, in autumn 2006, a second GII.1 norovirus outbreak occurred in Helsinki and the sequences determined were nearly identical to those in the Pirkanmaa outbreak. However, links between these outbreaks remain unknown.

Moreover, epidemiological findings suggested that raw vegetables and salads originating from a single provider were linked to the illness, although the analytical studies could not indicate either a specific food item as the vehicle of the outbreak or the exact date of exposure. Several lines of evidence supported the hypothesis that the outbreak was caused by food already contaminated before arriving at the kitchen. First, the outbreak occurred in several canteens simultaneously. Second, the kitchen staff either had no gastrointestinal symptoms or fell ill at the same time as the canteen users. Third, the epidemic curve was compatible with a point-source outbreak which is typical when contaminated food serves as the route of transmission. Deneen and colleagues have shown that in one third of restaurant outbreaks, no food worker could be implicated, thus implying that the food was contaminated before it arrived at the kitchen [11].

These epidemiological findings were, however, not supported by the microbiological results of food and environmental samples. The viral detection method used for food samples has been shown to be sensitive for finding noroviruses in lettuce. Despite much effort, no viruses were found in the vegetables. This may be due to less optimal sampling, problems in prolonged storage of fresh vegetables, and somewhat delayed laboratory investigations.

Workplace canteens are difficult settings for analytical epidemiological studies, because everybody eats almost the same foods. Relatively small cohorts might have decreased the power of analysis. These factors could explain the null results of the analysis of the canteen C cohort. In this outbreak investigation, the limited results of the analytical studies had no implications on the control measures because all the vegetable products were already recalled in the initial phase of the investigation. However, had the analytical results pointed to a suspected vegetable, it would have provided a basis for further trace-back investigations, which were difficult due to the complex farm-to-fork chain.

The investigation of this outbreak underscores the challenges associated with demonstrating norovirus contamination in food manufacturing industries. Vegetables and salads may become contaminated before harvest, during harvesting, packing, and storage, and during processing or preparation of cut products [12–14]. The present outbreak demonstrated that the commercial food chain is potentially hazardous and can result in a high number of cases within a short

time. The more concentrated the food preparation and the more complex the producer–supplier connections, the more difficult it can be to trace back to the farm. However, possibilities for successful trace back are crucial for providing a rational basis for the ultimate control measures.

Control measures in the case of a norovirus point-source outbreak focus on prevention because the outbreak is usually over before the investigation can begin. Thus, the aims of outbreak investigations are to prevent further spread and to reveal the mode of transmission and the vehicle in the outbreak. The latter can lead to measures aimed at preventing similar outbreaks, and requires enhanced traceability of foods potentially contaminated early in the food chain, including fresh produce.

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DECLARATION OF INTEREST

None.

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