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An outbreak of calicivirus associated with consumption of frozen raspberries

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SUMMARY

In April 1988, an outbreak of gastroenteritis occurred among employees in a large company in Helsinki, Finland. A retrospective cohort study, using a self-administered questionnaire, was carried out to ascertain the cause and extent of the outbreak. To meet the case definition, employees had to have had diarrhoea and/or vomiting since 2 April, 1998. A subanalysis was made in the biggest office, consisting of 360 employees, of whom 204 (57%) completed the questionnaire. Of these 108 (53%) met the case definition. Employees who had eaten raspberry dressing were more likely to meet the case definition than those who had not (Attack Rate (AR) 65% versus AR 18% Relative Risk, (RR) 3·7, 95%, Confidence Intervals (CI) 2·0–6·7). Four stool specimens obtained from affected kitchen staff who had all eaten the raspberry dressing and who had all become ill simultaneously with the employees were positive by polymerase chain reaction (PCR) for calicivirus. The data suggest that the primary source of the outbreak was imported frozen raspberries contaminated by calicivirus.

INTRODUCTION

The major causes of outbreaks of non-bacterial gastroenteritis throughout the world are small round viruses (e.g. calici-, astro- and enteroviruses), including those that used to be known as Norwalk agent and Norwalk-like viruses [1–5]. Transmission of small round viruses by water and by various foods, including salads, bakery products, fresh-cut fruits, chicken, oysters, and mussels, has been documented [6–20]. Except in water-borne outbreaks and those associated with shellfish, viral contamination of food has usually been attributed to infected food handlers. The recent development of novel molecular methods for detecting and differentiating small round viruses has allowed the diagnosis of viral gastroenteritis with increased sensitivity and specificity [21, 22]. In Finland, polymerase chain reaction (PCR) diagnostics for calicivirus were begun in autumn 1997. The system for surveillance and reporting of food- and water-borne outbreaks in Finland was reorganized in autumn 1997, and this has led to closer interaction between local health authorities and the National Public Health Institute when investigating outbreaks. On several occasions, imported frozen raspberries have been suspected to be the source of the outbreaks, especially when caliciviruses have been detected in the stool specimens of ill persons. Previously, no outbreak of calicivirus attributed to frozen berries has been published. We report the first outbreak of gastroenteritis caused by calicivirus known to be associated with consumption of frozen raspberries imported into Finland.

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MATERIALS AND METHODS

Background

On 6 April 1998, the Environmental Centre of Helsinki was informed of a cluster of employees with gastroenteritis in the offices of a large company in Helsinki. Most of the employees affected had lunched at their local canteens. The company's central kitchen delivers 1200–1300 lunch portions daily to the local canteens of 59 different offices of the company located in the Helsinki metropolitan area. Luncheon is served from 11.00 to 13.00 hours. The menu usually includes a warm dish, salads and a dessert, as well as choices for those on special diets. The first employees became ill in the evening of 3 April 1998. Most cases occurred on 4 April, but new cases were still occurring on 6 April. The staff of the central kitchen included 16 workers, 7 of whom had become ill simultaneously with the company employees and with similar symptoms.

Epidemiological investigation

To determine the cause and extent of the outbreak, a cohort study of the company employees taking lunches at their local canteens was carried out retrospectively. The questionnaires were distributed with lunch portions at all local canteens of the 59 offices on 8 April 1998. All employees who had lunched at their local canteens during the week from 30 March 1998 to 3 April 1998 were asked to fill in the questionnaire. In this way information was gathered about the onset, duration and characteristics of symptoms among employees since 2 April 1998, the foods eaten on 2–3 April, demographics, subsequent illness in family members and travel. To meet the case definition, employees had to have had diarrhoea and/or vomiting on or after 2 April 1998. Univariate statistical analysis of the categorical data was performed using the χ^2 test and Fisher's exact test, as appropriate. Confidence intervals (CI) for relative risks (RR) were calculated using EpiInfo.

Laboratory and environmental investigation

Stool specimens were obtained from 4 affected employees (collected 8 April) and 5 affected kitchen workers (collected 6–7 April) and cultured for salmonella, shigella, campylobacter, yersinia, *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus*. Samples of leftover food items served on 2 and 3 April 1998 taken by the health officer of the Environmental Centre were cultured for *Cl. perfringens*, *Staphylococcus* sp. *Salmonella* sp. and *B. cereus*. In addition, kitchen workers were interviewed regarding the timing and methods of food preparation.

In the PCR test, stool samples were first subjected to RNA extraction using a commercial phenol-containing Tripure reagent (Boehringer–Mannheim), then subjected to reverse transcription PCR for calicivirus genogroups I and II, respectively as described by Le Guyader and colleagues [22]. The agarose gel findings were confirmed by hybridization method and also by amplicon sequencing.

Raspberries were melted and washed with phosphate buffer. The supernatant was separated by low speed centrifugation. Concentration was achieved by either ultracentrifugation or by filtration through a positively charged nylon membrane [23]. As a control, raspberry samples were spiked with calicivirus and then submitted to similar recovery attempts.

RESULTS

Epidemiological investigation

Of the 741 employees (57% of a total of 1296) who completed the questionnaire, 509 (69%) reported gastrointestinal symptoms since 2 April. Because of the large number of employees, a more detailed analysis of the food exposures was made among the employees of the biggest office of the company, which included 360 employees, 204 (57%) of whom completed the questionnaire. Of these, 108 (53%) met the case definition.

Of the 106 cases for whom the time of onset of symptoms was available, 76 (72%) had become ill within 2 days (Fig. 1). The first had symptoms at 01·00–02·00 hours on 3 April. The median time between lunching on 2 April and the onset of symptoms was 41 h (range 12–119 h). The symptoms most commonly reported were diarrhoea (94%), abdominal cramps (73%), nausea (64%), fatigue (62%), headache (52%), fever (46%) and vomiting (45%). The median duration of diarrhoea was 48 h (range, 2–120 h) and among 67 cases (69%) it lasted more than 24 h. Only one case reported having seen blood in the faeces. No-one was hospitalized.

Employees who reported that they had eaten raspberry dressing were almost four time as likely to

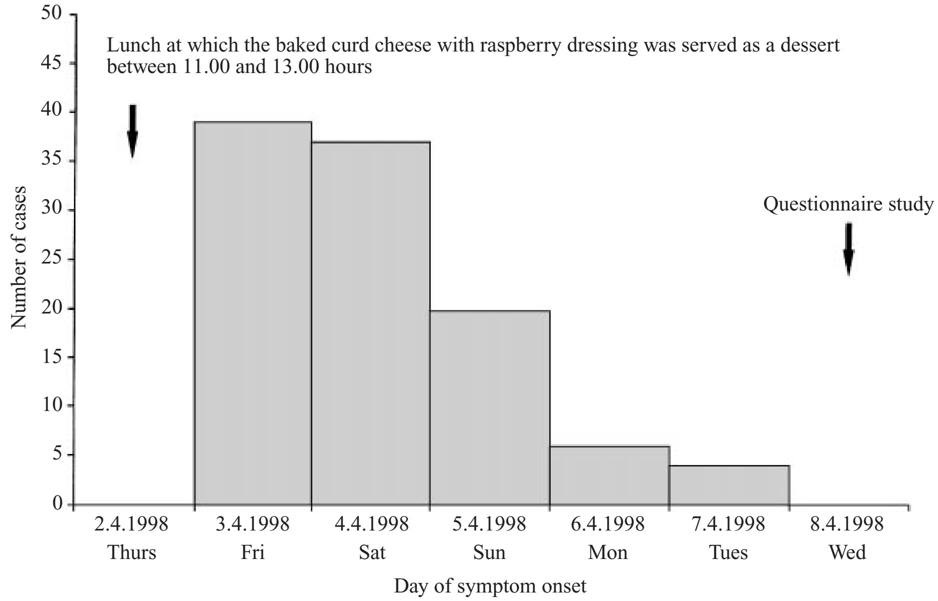

Fig. 1. Cases of gastroenteritis by date of symptom onset.

Table 1. *Gastroenteritis among employees, by food they reported to have eaten on 2 and 3 April 1998*

	Ate			Did not eat					
Food	No. cases*	Total	AR (%)	No. cases*	Total	AR (%)	RR	95% CI	*P* value
Cabbage soup	53	96	55	55	108	51	1·1	0·8–1·4	0·64
Tomato	56	115	49	52	89	58	0·8	0·6–1·1	0·22
Baked curd cheese	97	149	65	11	55	20	3·3	1·9–5·6	0·00000003
Raspberry dressing	99	153	65	9	51	18	3·7	2·0–6·7	0·00000001
Meat soup	16	24	67	92	180	51	1·3	1·0–1·8	0·22
Herrings	69	118	58	39	86	45	1·3	1·0–1·7	0·09
Herrings without lactose	3	6	50	105	198	53	0·9	0·4–2·2	1·00
Ham salad	62	109	57	46	95	48	1·2	0·9–1·5	0·29
Ham salad, light	4	5	80	104	199	52	1·5	1·0–2·4	0·37
Avocado mixture	5	12	42	103	192	54	0·8	0·4–1·5	0·61
Cheese salad	22	37	59	86	167	51	1·2	0·9–1·6	0·49
Mysli	11	26	42	97	178	54	0·8	0·5–1·2	0·34
Cheese, Edam	88	158	56	20	46	43	1·3	0·9–1·8	0·20
Cucumber	79	147	54	29	57	51	1·1	0·8–1·4	0·83
Baked curd cheese without lactose	10	20	50	98	184	53	0·9	0·6–1·5	1·00
Dream dessert without lactose	2	4	50	106	200	53	0·9	0·4–2·5	1·00
Vegetable soup	22	41	54	86	163	53	1·0	0·7–1·4	0·94
Baked potatoes	66	130	51	42	74	57	0·9	0·7–1·2	0·50
Mushroom salad	22	48	46	86	156	55	0·8	0·6–1·2	0·34
Mushroom salad without lactose	3	8	38	105	196	54	0·7	0·3–1·7	0·48
Salad-cucumber-tomato	58	114	51	50	90	56	0·9	0·7–1·2	0·60
Roast mixture, light	9	17	53	99	187	53	1·0	0·6–1·6	0·80
Blueberry soup	21	45	47	87	159	55	0·9	0·6–1·2	0·43

* Employees who had lunched at their local canteen during the week from 30 March 1998 to 3 April 1998 and reported to have had diarrhoea and/or vomiting since 2 April 1998.

meet the case definition as those who did not (AR 65% versus AR 18%, RR 3·7, CI 95%, 2·0–6·7; $P <$ 0·01) (Table 1). Of the other foods eaten, only the baked curd cheese was implicated. If we assume that those with onset of symptoms after 4 April are not primary cases and so take into account only the 76 case persons with onset of symptoms on 3 and 4 April, the results were the same with regard to eating

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raspberry dressing (AR 12% versus 46%, RR 3·9, 95% CI 1·8–8·4). Of the 108 cases, 17 (16%) reported subsequent gastrointestinal illness in their family members, but only 5 (5%) of the non-cases ($P =$ 0·028). Holidaying at home or abroad was not associated with the illness.

Laboratory and environmental investigation

Four of the nine faecal specimens were positive for calicivirus by PCR and all belonged to genogroup 2. These four positive specimens were obtained from affected kitchen workers who had all eaten the lunches delivered to the local canteens, including the raspberry dressing, and all became ill on 3–5 April 1998. Of the five negative specimens, one was obtained from an ill kitchen worker and the rest from employees from whom no questionnaire was available. These specimens were all negative for astrovirus by PCR and also in electron microscopy. The cultures were also negative for all other bacterial pathogens, except those from two kitchen workers, from whom *Clostridium perfringens* was isolated. The number of these bacteria was 10^3–10^4 in one case, and 10^5–10^6 in the other. The last-mentioned strain produced enterotoxin.

The baked curd cheese had been made by keeping the mixed ingredients at 120–140 °C for 1 h, but the dressing from imported frozen raspberries had not been heated. The raspberry dressing was served with the baked curd cheese and 97% of the employees had eaten both together.

Specimens of all food items served on 3 April and, of those served 2 April only cabbage soup were tested for bacterial pathogens. Mushroom salad gave the only positive result, *S. aureus* (7800 cfu/g) but no *S. aureus* toxin was detected in the sample.

Attempts to isolate genomes of caliciviruses from the raspberries were unsuccessful. The purification and concentration methods applied to the spiked raspberry samples indicated that a 10- to 100-fold inhibition persisted as compared with samples prepared in PBS buffer.

DISCUSSION

Our study indicated an epidemiological association between gastroenteritis and eating frozen raspberries contaminated by calicivirus.

Symptoms including vomiting, diarrhoea, abdominal cramps and fever, and the occurrence of secondary cases were compatible with small round viruses including calicivirus. The illness was strongly associated with consumption of dressing prepared from imported frozen raspberries. In addition, calicivirus was detected in the stools of four affected persons. All those with positive stool specimens were kitchen staff, who were sampled earlier than the other company employees. However, none of the kitchen staff was ill before the start of the outbreak and all had eaten the raspberry dressing, suggesting that they had been infected from the same source as the company employees rather than been the source of the outbreak.

The shape of the epidemic curve is consistent with a point source rather than a continuing source of infection. More than 70% of those affected fell ill within 48 h (the maximum incubation time for calicivirus infection). In several persons, symptoms appeared more than 48 h after the start of the outbreak. This suggests that person-to-person transmission, characteristic of calicivirus infection, occurred not only in families but also between employees. However, these late-onset symptoms suggest that some of those affected were not connected with this outbreak or could not accurately recall the time of onset of their symptoms.

Freezing allows long survival of viruses in berries. In Finland, domestic raspberries are scarce, especially for freezing, and frozen raspberries are often imported from foreign countries. We could not trace the suspected raspberries from several epidemics occurred in Finland to a single lot. However, we know that all were imported from East European countries. To our knowledge, there are at least two ways in which the raspberries could have become contaminated with calicivirus. Caliciviruses have been detected in river water in Europe [23]. Contaminated water might have been used for irrigation of raspberries in the field, or alternatively, for spraying the berries just before they were frozen.

Contaminated berries have previously been reported to transmit viruses other than small round viruses. Recently, frozen strawberries have caused two widespread outbreaks of hepatitis A in the USA [24, 25]. In addition, one outbreak of hepatitis A in Scotland has been linked to the consumption of mousse prepared from frozen raspberries [26]. Increased awareness of the possibility that berries transmit viruses and improved diagnostics to assess viral aetiology might explain the association between calicivirus outbreaks and raspberries detected in Finland. On the other hand, the increased occurrence may also be real. Globalization of food markets is a worldwide phenomenon, caused, among other things, by the creation of the World Trade Organization [27, 28]. Importation of food has also increased in Finland since joining the European Union.

Unfortunately, we could not detect caliciviruses from the raspberries by PCR. Hitherto, the only foods in which caliciviruses have been successfully detected are shellfish [21, 29]. This is probably due to the high concentration of the viruses in shellfish [21, 29]. The difficulty in detecting viruses in berries may be connected with the various inhibitors present in the berries. There is a need for better techniques to confirm the role of viruses as causative agents in foodborne outbreaks.

Our paper is the first report of an association between frozen berries and an outbreak of viral gastroenteritis. Because of the findings of this report and of suspicions in several similar outbreaks in Finland, the national food control authorities have temporarily recommended institutional kitchens not to serve unheated dishes prepared from frozen berries. It is possible that berries are more a common vehicle for transmission of viral gastroenteritis than has hitherto been suspected. Future epidemiological studies, in combination with novel molecular techniques, will assess the significance of berries in transmitting small round viruses.

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