

## DETECTION OF HUMAN NOROVIRUS FROM FROZEN RASPBERRIES IN A CLUSTER OF GASTROENTERITIS OUTBREAKS

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We describe a cluster of norovirus outbreaks affecting about 200 people in Southern Finland in September and October 2009. All outbreaks occurred after consumption of imported raspberries from the same batch intended for the catering sector. Human norovirus genotype GI.4 was found in frozen raspberries. The berries were served in toppings of cakes in separate catering settings or mixed in curd cheese as a snack for children in a daycare centre. The relative risk for consumption of the berry dish was 3.0 ( $p \leq 0.05$ ) at the daycare centre. Human norovirus GI.4 was also detected in samples from two patients, and in berries. Both shared identical partial capsid sequences. Based on the results of epidemiological, trace-back and laboratory investigations it was concluded that one particular batch of frozen raspberries was the source of all outbreaks.

### Introduction

Human norovirus is a common cause of outbreaks of acute gastroenteritis worldwide [1]. Food-borne outbreaks caused by contaminated shellfish occur commonly, but fresh products, especially raspberries have also been found to be the vehicle [2, 3, 4]. In Finland, from 1997 to 1999, several norovirus outbreaks were linked to imported frozen raspberries [2,5]. It still remains unclear how the berries were contaminated, but it seemed to have occurred already in the countries of origin. Pre-harvest irrigation or hygiene failures during harvest/freezing have been suggested as possible sources of contamination [6]. A proper heating of frozen raspberries prior to consumption has been recommended by the Finnish Food Safety Authority since 2000 but it is occasionally neglected.

Here we describe a trace-back investigation in a cluster of three food-borne norovirus outbreaks linked to consumption of imported raspberries affecting about 200 people in Southern Finland in September and October 2009. The epidemiological investigation was performed of one of the outbreaks, at a daycare centre.

### Outbreak in the daycare centre

A curd cheese dish mixed with raspberries (originally frozen) was served without heating the berries and eaten by about 90 persons (majority children, less than 7 years old) at a daycare

centre on 2 October 2009 at 2-2.30 pm. On Saturday evening, 3 October, more than 20 of the 90 persons started symptoms of vomiting and diarrhoea (Table 1). The food inspection authorities were informed about the outbreak on 6 October and started an epidemiological investigation. No samples of the dish were available for investigation but the remaining frozen raspberries were sent for bacteriological and virological examination on 7 October. Also samples from patients were collected, and questionnaires were distributed to the children's parents and the personnel on 7 October to investigate the possible source of the outbreak. The outbreak occurred at a daycare centre in a city of 100,700 inhabitants in Southern Finland.

### Epidemiological analysis

Questionnaires were obtained from 69 people at the daycare centre. A case was defined as a person who was working, or at daycare at the daycare centre, and fell ill with vomiting and diarrhoea between 2 and 5 October 2009. A two-by-two table for consumption of berries was performed (a cohort study). A chi-squared test was used to calculate the statistical significance.

Most cases (45/46, 98%) had eaten berries. The epidemic curve shows a point-source pattern with some secondary cases (Figure). The incubation period was determined at 32.5 h (range 14-76) and the mean duration of symptoms was 22.4 h (range 1-72). Based on

**TABLE 1**  
Symptoms among cases daycare centre, norovirus outbreak, Finland, October 2009 (n=46)

Symptoms	N (%)
Vomiting	42(91)
Nausea	33 (72)
Stomach pain	30 (65)
Diarrhoea	17 (37)
Fever	12 (26)
Headache	10 (22)

a cohort study, those who ate berries were 3.0 times (relative risk) more likely to develop disease than those who did not ( $p \leq 0,05$ ).

#### Food and patient samples from three outbreak settings

Three samples of frozen raspberries obtained from three outbreak settings (Table 2) and two samples from the wholesaler's stock (total batch size 20,000 kg) were analysed for norovirus at the laboratory of the Department of Virology, Helsinki University Central Hospital (HUSLAB), Helsinki, Finland. The raspberries, packed in bags of 2.5 kg, originated from Poland. Patient samples from two outbreaks were sent for norovirus analysis to HUSLAB and

sequencing and genotyping was performed at the laboratory of the Finnish Institute of Health and Welfare. A norovirus real-time RT-PCR was performed targeting the polymerase-capsid gene junction [7]. The sequence analysis was performed on the polymerase and capsid region with primer-pairs MJV12-RegA and SKF-1-SRI-3, respectively [8-10]. The expected lengths of amplicons were 320 and 240bp, respectively. The accession numbers for the norovirus sequences are GU188278 (capsid; berries) and GU 188279 and GU188280 (capsid and polymerase; patient).

### FIGURE

Epidemic curve, norovirus outbreak, day-care center, Finland, October 2009 (n=46)

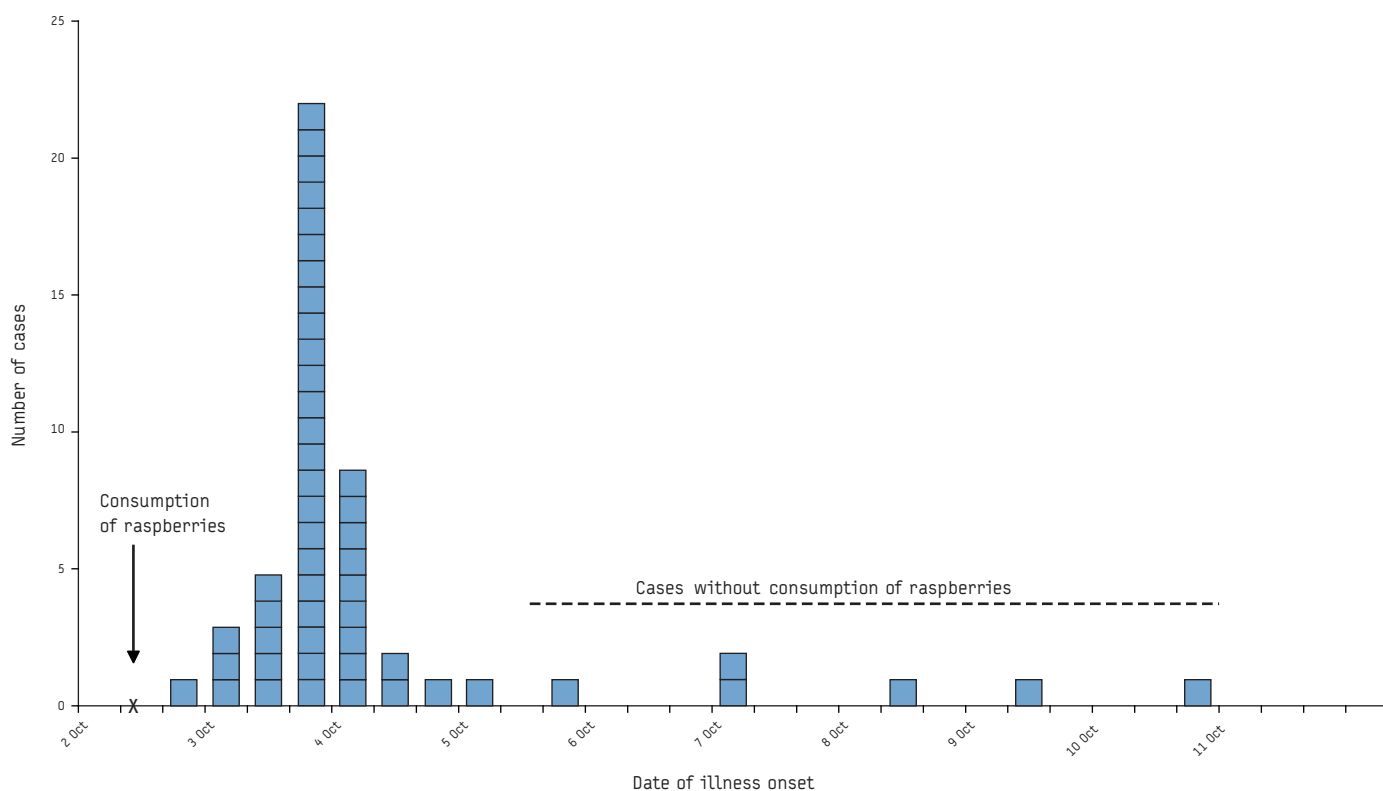


TABLE 2

Onset of outbreak, number of cases among exposed and detected norovirus, by place, Finland, September-October 2009

Place (provider of food)	Start date	Cases/exposed (estimation)	Virus in raspberries	Virus in patients
Restaurant (catering)	26 September	(15/30)	1/1 NoV GI.4 (Cp 34,8)	0/0
Daycare centre (prepared on site)	2 October	46/90	1/1 NoV GI (Cp 40,1 1:10 37,0)	2/3 NoV GI.4
Cafeteria (catering)	3 October	(15/30)	1/1 NoV GI (Cp 37,8)	1/2 NoV
Raspberries from wholesaler's stock	NA	NA	0/2	NA

Cp = crossing point -value in norovirus real-time RT-PCR (LightCycler, Roche); NoV: norovirus; NA: not applicable.

### Microbiological findings

In total, norovirus GI was detected in three of five raspberry samples analysed by norovirus real-time RT-PCR. In one case the concentration of virus was high enough to allow exact genotyping and the virus could be identified GI.4 by conventional RT-PCR and sequence determination. In the two patient samples available for genotyping, norovirus GI.4 was detected. The viruses in berries and patients showed identical nucleotide sequences in the short 181 bp-capsid gene region. A positive polymerase RT-PCR result could only be amplified from patient samples.

In addition to the three outbreaks described, several smaller outbreaks involving only few cases (e.g. bakery, bank) that were most likely berry-related, were reported to the local food health authorities between 26 September and 9 October, but no samples were obtained for virological investigation. Taken together, about 200 people were affected in all these outbreaks.

### Discussion and conclusions

Strong laboratory evidence supported the epidemiological findings that imported raspberries were the source of the norovirus outbreaks, since the identical genotype was detected in samples from berries and patients. The outbreaks occurred outside of the norovirus outbreak season that usually occurs from December to May in Finland. The detection of GI.4 virus is in line with a large study of norovirus outbreaks in which the proportion of non-GII.4 outbreaks was found to be higher in food-borne outbreaks, whereas GII.4 outbreaks were mostly linked to person-to-person transmission [11].

The berries that caused the outbreaks were likely to contain a considerable number of viruses, since they were detected without prior concentration of the samples. While the present real-time RT-PCR method is quite sensitive, the positivity in foods is mostly weak, partly due to PCR inhibitors. To determine the viral genotype with the less sensitive conventional RT-PCR is therefore challenging. In this study, a short sequence in a capsid gene region, not normally used for genotyping could be determined in berries, independently from the patient sample analysis.

Our findings highlight the importance of routine investigations of food samples for viral pathogens in addition to bacterial analyses. So far, all our virus findings in foods have been directly linked to outbreaks. In spite of analysing several samples of the same batch of raspberries epidemiologically linked to human cases, norovirus could not be detected in all samples. This could be due to an uneven distribution of viruses in the berries.

The norovirus gastroenteritis outbreaks rapidly died out, after the contaminated batch was withdrawn from the market. Furthermore, the Finnish authorities issued an alert through the Rapid Alert System for Food and Feed (RASFF) on 20 October to inform other European Union countries of the outbreaks caused by norovirus-contaminated raspberries. It is noteworthy that a month earlier, in August, another food-borne outbreak in east Finland was epidemiologically linked to crushed frozen raspberries also imported from Poland. No viruses were found in the berries, but genotype GI.4 norovirus was found in the patients.

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