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Raw grated beetroot linked to several outbreaks of sudden-onset gastrointestinal illness, Finland 2010

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SUMMARY

In 2010, 7/44 (16%) reported foodborne outbreaks in Finland were linked with raw beetroot consumption. We reviewed data from the national outbreak registry in order to hypothesize the aetiology of illness and to prevent further outbreaks. In the seven outbreaks, 124 cases among 623 respondents were identified. Consumption of raw beetroot was strongly associated with gastrointestinal illness (relative risk 8·99, 95% confidence interval 6·06–13·35). The illness was characterized by sudden onset of gastrointestinal symptoms; the median incubation time was 40 min and duration of illness 5 h. No common foodborne pathogens or toxins were found in either clinical or beetroot samples, but all tested beetroot samples were of poor quality according to total bacterial counts. Beta-haemolytic *Pseudomonas fluorescens* was detected in several beetroot samples but its effect on human health is unknown. No outbreaks were reported after the Finnish Food Safety Authority Evira advised against serving raw beetroot in institutional canteens.

Key words: Beetroot, gastrointestinal illness, meta-analysis, *Pseudomonas fluorescens*.

INTRODUCTION

In Finland, municipal authorities notify suspected foodborne and waterborne outbreaks to a national online registry developed and maintained by the National Institute for Health and Welfare and the Finnish Food Safety Authority Evira. Notification is mandatory and involves outbreaks with >5 non-family members with similar symptoms or only one person with a suspicion of a serious illness, i.e. botulism or invasive listeriosis. On average, 70 foodborne and waterborne outbreaks are notified to the registry per year. The purpose of the registry is to promote surveillance and outbreak

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investigations, and facilitate reporting at national and international levels, including providing annual reports to the European Food Safety Authority.

In October 2010, two notifications submitted to the foodborne and waterborne outbreak registry (FWO registry) stated rapid onset of illness with vomiting as a frequent symptom in affected persons and a possible link between gastrointestinal illness and consumption of raw grated beetroot.

We identified similar outbreaks from the FWO registry for 2010 and reviewed the epidemiological, microbiological and traceback investigation data in order to hypothesize the aetiology of the illness and to prevent further outbreaks.

MATERIALS AND METHODS

Epidemiological investigation

We reviewed outbreak notifications and investigation reports from the FWO registry for 2010. Outbreaks involving sudden onset of symptoms and a suspected link to beetroot consumption were included in this study. For outbreaks that fulfilled these criteria, we requested original questionnaires, datasets and details on clinical and food microbiological analyses, as well as traceback investigations from the municipal authorities.

Based on the frequency of symptoms reported in the investigation reports, we generated a common case definition. A case was defined as anyone reporting nausea or stomach pain within 24 h of consuming lunch at one of the canteens investigated in the outbreaks selected for this study. We used this case definition when calculating the time of onset (in minutes) and duration (in hours) of illness. For each cohort study, we extracted data on exposure in cases and non-cases to the food items served in the respective canteens during the relevant period, and calculated attack rates (AR, %) and relative risks (RR) with 95% confidence intervals (CI) for each exposure using Jewell's small sample adjustment [1, 2] to handle cells with no observations. Fisher's exact test was used for significance testing in univariate analyses. Data were analysed using Stata v. 10.1 (Stata Corporation, USA). To calculate a summary estimate for an association between exposure to raw grated beetroot and gastrointestinal illness based on all available datasets, we used meta-analysis with a random-effects model according to DerSimonian and Laird [3, 4] as provided in R package METAFOR [5].

Microbiological and chemical investigations

Data on microbiological analyses from food items suspected of being linked to the illness were extracted from the outbreak investigation reports. In conjunction with sensory evaluation, culture for any spoilage bacteria was performed and deemed of 'poor quality' if total bacterial counts exceeded 100×10^6 colony-forming units (c.f.u.)/g. Reports also provided results on detection of specific pathogens including *Salmonella* (negative/25 g), *Campylobacter* (negative/25 g), *Listeria monocytogenes* (negative/25 g), *Bacillus cereus* (<100 c.f.u./g), *Staphylococcus aureus* (<100 c.f.u./g) and *Clostridium perfringens* (<10 c.f.u./g).

Beetroot samples were stored in refrigerators at local laboratories before being sent to the Finnish Food Safety Authority Evira, where they were stored at $-70\,^{\circ}$C until further analyses. Samples were analysed for content of nitrate and nitrite (mg/kg). Due to earlier investigations of sporadic food poisonings in 2002 in Porvoo municipality, Finland, where β-haemolytic *Pseudomonas fluorescens* strains were suspected to be a cause of gastrointestinal illness with sudden onset of symptoms, available samples were cultured on bovine blood agar plates, incubated at 22 °C for 24–48 h; suspected colonies of *P. fluorescens* were confirmed by positive oxidase test, positive haemolysis test, growth at 5 °C/48 h, no growth at 42 °C/48 h and API NE test.

RESULTS

Review of outbreak notifications and investigation reports

In 2010, 44 foodborne outbreaks were reported to the FWO registry; we identified seven (16%) outbreaks where rapid onset of symptoms and exposure to raw or raw grated beetroot was reported. These outbreaks occurred at institutional canteens (one secondary school, six companies) in five different cities throughout the country. The outbreaks occurred between January and November 2010; 5/7 during autumn. Four canteens provided information on estimated daily throughput of lunch guests, ranging from 158–340 (Table 1). All investigations conducted by the municipal authorities had been performed as retrospective cohort studies.

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Table 1. *Descriptive epidemiology of outbreaks of sudden-onset gastrointestinal illness, Finland 2010*

Location	Date	Estimated daily canteen throughput	Questionnaire respondents			Cases				
			N	Median age, years (range)	Female (%)	*N*	Median age, years (range)	Female (%)	Median incubation time (min)*	Median duration (h)†
A	January	158	35	43 (27–65)	42·9	8	30 (27–58)	62·5	60	2·50
B	February	200	21	43 (21–60)	66·7	13	44 (26–60)	76·9	60	5·50
C	September		47	50 (21–62)	87·2	16	51 (36–61)	100·00	15	4·00
D	October		250	41 (23–62)	56·8	34	41 (23–54)	73·5	60	8·00
E	October	340	172	16 (15–59)	87·2	33	17 (15–40)	87·9	30	5·00
F	October		54	49 (24–61)	79·6	13	52 (31–60)	84·6	40	2·25
G	November	300	44	39 (21–61)	72·7	7	39 (21–60)	85·7	90	60·00
Total			623	36 (15–65)	70·1	124	38 (15–61)	82·3	40	4·75

* Based on data provided by 103/124 cases.
† Data provided by 105/124 cases.

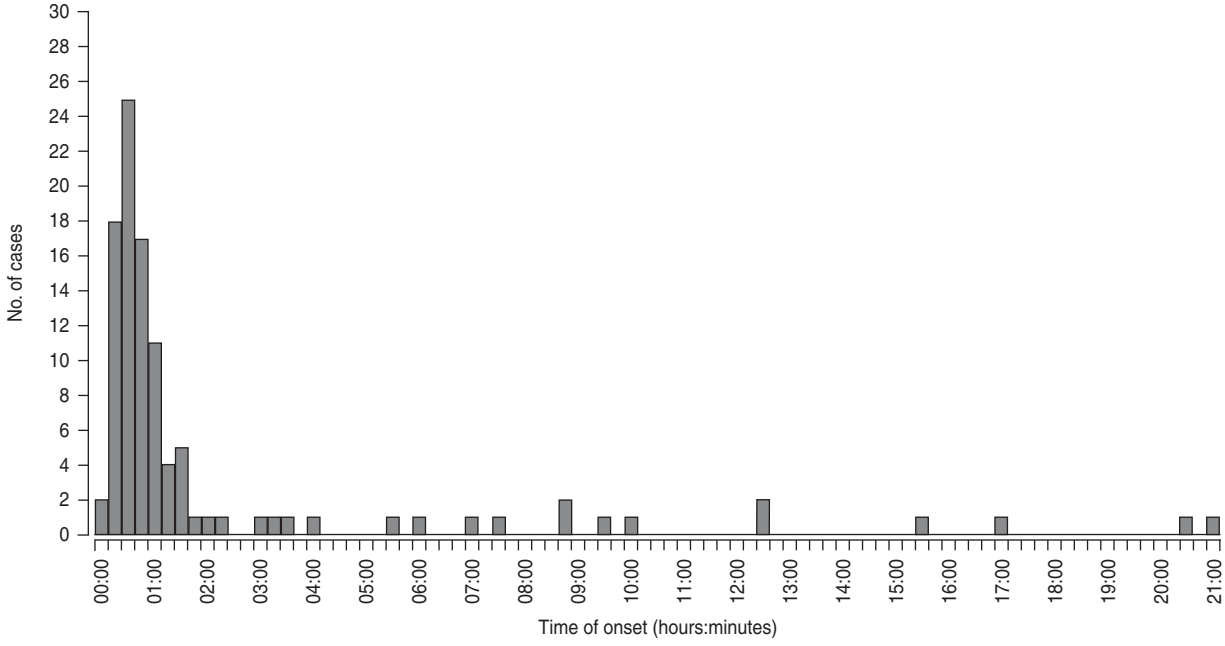

Fig. 1. Cases ($n = 103$) by time of onset of illness in outbreaks of sudden-onset gastrointestinal illness, Finland 2010. Data based on 5/7 outbreaks (in two outbreaks, data regarding onset time were provided only in hourly units).

Description of the cases and illness

We identified 623 respondents in the studied outbreaks (range 21–250 per outbreak) and 124 cases met the common case definition (range 7–34 per outbreak). Attack rate per outbreak varied between 14% and 62%. Study respondents and cases were predominantly females (70% and 82%, respectively), median age for respondents and cases was similar (36 and 38 years, respectively; Table 1).

Of the cases, nausea was the most frequent symptom (109/124, 88%), followed by stomach ache (80/124, 65%), vomiting (39/124, 31%) and diarrhoea (26/124, 21%). No case was hospitalized as a result of symptoms. Median onset time of illness was 40 min from the meal (range <5 min to 21 h, Fig. 1). Median duration of illness was 5 h (range 5 min to 8 days, Table 1).

Investigation reports provided results from microbiological analyses of stool or vomitus samples in 4/7 outbreaks. Stool samples from nine cases were negative for pathogenic bacteria (*Salmonella*, *Campylobacter*, *Yersinia*, *Shigella*, *Cl. perfringens*, *B. cereus*, *S. aureus*). In PCR testing for norovirus and

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Table 2. *Exposure to raw grated beetroot in outbreaks of sudden-onset gastrointestinal illness, Finland 2010*

	Exposed to raw beetroot			Not exposed to raw beetroot					
Location	Total	Cases	AR, %	Total	Cases	AR, %	RR	95% CI	*P**
A	14	8	57·14	19	1†	5·26	10·90	(1·53–77·15)	0·001
B	13	12	92·31	8	1	12·50	7·38	(1·17–46·50)	0·001
C	24	15	62·50	21	1	4·76	13·13	(1·89–91·12)	0·000
D	41	20	48·78	209	14	6·70	7·28	(4·02–13·21)	0·000
E	37	25	67·57	134	8	5·97	11·32	(5·58–22·98)	0·000
F	16	11	68·75	38	2	5·26	13·06	(3·26–52·38)	0·000
G	21	7	33·33	15	1†	6·66	5·00	(0·68–36·50)	0·060
Pooled analysis	166	98	59·03	444	28	6·30	9·36	(6·40–13·69)	0·000
Meta-analysis‡	166	98	59·03	444	28	6·30	8·99	(6·06–13·35)	

AR, Attack rate; RR, relative risk; CI, Confidence interval.
* Fisher's exact test.
† Jewell's small sample adjustment applied.
‡ DerSimonian & Laird.

astrovirus, and electron microscopy, norovirus was detected in one stool sample (1/4 tested in outbreak A). Two cases from outbreak E provided vomitus samples that were submitted to the Finnish Food Safety Authority Evira's food microbiology laboratory for analysis of staphylococcal enterotoxins (A, B, C, D, E), which could not be detected.

Analytical epidemiological analysis

In 6/7 outbreaks, raw grated beetroot was the only food item in the univariate analysis significantly associated with gastrointestinal illness with rapid onset (Table 2). In outbreak G, no single food item including raw beetroot reached significance in the univariate analysis. In outbreak B, in addition to raw beetroot, another food item was suspected (fresh salad, RR 4·80, 95% CI 0·79–29·94, $P=0·014$). However, stratification supported raw grated beetroot as the source of the illness since of the 13 cases in outbreak B, 11 had consumed both raw beetroot and another fresh salad, and only one had solely eaten the fresh salad item.

We found a statistically significant association between consumption of raw grated beetroot and illness in the pooled analysis and meta-analysis (RR 9·36, 95% CI 6·40–13·69 and 8·99, 95% CI 6·06–13·35, respectively; Table 2, Fig. 2).

Traceback investigation

The investigated canteens refrigerated ready-peeled, rinsed and packed beetroot for 5–25 days before serving; the beetroot was delivered from two different peelers and three different domestic producers without any connection in time or geography. The beetroot involved in spring outbreaks (A, B) was harvested during summer 2009 whereas the beetroot involved in autumn outbreaks (C–G) was harvested during summer 2010. In 5/7 outbreaks beetroot samples were cultured for any spoilage bacteria. All these samples were of poor quality according to total bacterial counts but no pathogenic bacteria could be detected. The result of a sensory evaluation was acceptable in 2/2 tested samples (outbreaks D and E). Nitrate levels were considered normal in the two samples tested (1420 and 1450 mg/kg, respectively). *P. fluorescens* was detected in high amounts in 4/5 beetroot samples (Table 3).

DISCUSSION

During 2012, one sixth of the outbreaks notified to the Finnish FWO registry were linked to consumption of raw grated beetroot. In separate univariate analyses and in meta-analysis, the association between consumption of raw beetroot and development of gastrointestinal illness was strong. The consistency of illness characteristics across the seven studied outbreaks, separated in time and geography, also supports the causal relationship between consumption of raw beetroot and gastrointestinal illness. No common foodborne pathogen could be identified. The Finnish Food Safety Authority Evira advised against serving raw beetroot in public settings based on the preliminary findings of the present study in

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Table 3. *Laboratory analyses and traceback investigations of beetroot samples collected in outbreaks of sudden-onset gastrointestinal illness, Finland 2010*

Location	Outbreak month	Microbiological quality*	*Bacillus cereus Staphylococcus aureus Clostridium perfringens Listeria monocytogenes Salmonella Campylobacter*†	Biochemical analysis‡	*Pseudomonas fluorescens*‡ Detection threshold $>10^4$c.f.u./g	Storage days for beetroot between peeling and serving§
A	January	—	Not detected	Nitrate 1420 mg/kg Nitrite not detected	—	10
B	February	Poor	Not detected	Nitrate 1450 mg/kg Nitrite 157 mg/kg	$9{\cdot}0 \times 10^6$c.f.u./g	25
C	September	Poor	Not detected	—	Not detected	6
D	October	Poor	—	—	$1{\cdot}5 \times 10^6$c.f.u./g	8
E	October	Poor	Not detected	—	—	18
F	October	Poor	Not detected	—	$6{\cdot}5 \times 10^6$c.f.u./g	5
G	November	—	—	—	$4{\cdot}0 \times 10^6$c.f.u./g	5

* Poor if total bacterial count $>100 \times 10^6$c.f.u./g.
† Data collected from outbreak investigation reports submitted to the foodborne and waterborne outbreak registry.
‡ Analyses performed at the food microbiology and chemistry laboratories of Finnish Food Safety Authority Evira.
§ Data collected from the kitchens and production plants (interviews, consignment notes, internal records).

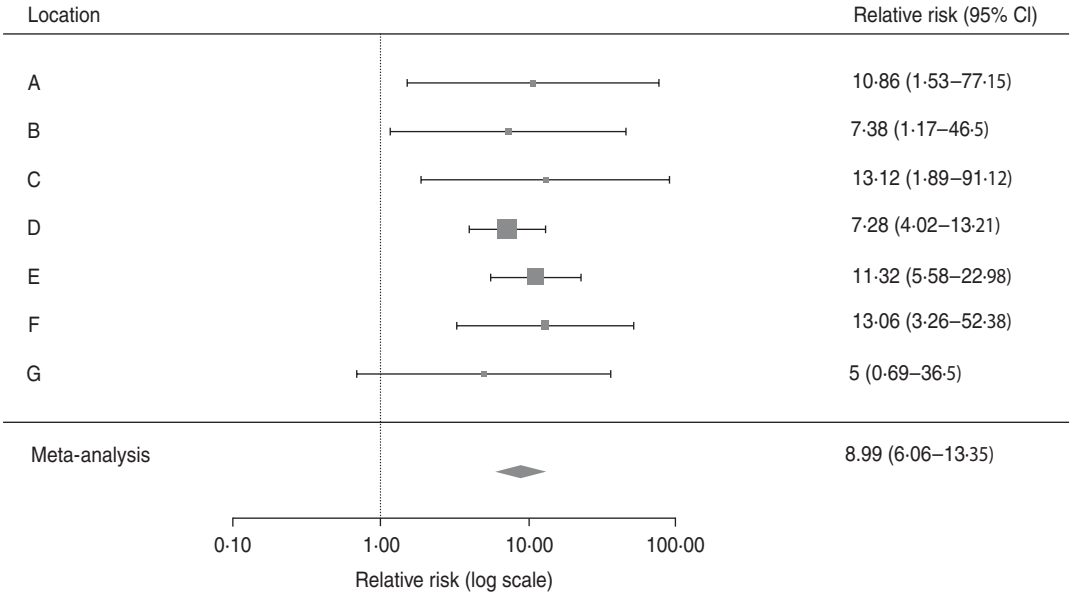

Fig. 2. Relative risk of illness following consumption of raw grated beetroot in outbreaks of sudden-onset gastrointestinal illness, Finland 2010. CI, Confidence interval.

November 2010; since then, no similar outbreaks have been notified.

In this study, we used the FWO registry to identify outbreak notifications and reports with similar characteristics. Outbreak notification is mandatory for municipal authorities and is conducted with the purpose of promoting surveillance activities as well as outbreak investigations at the municipal and national levels. Hence the FWO registry is believed to be a nationally exhaustive database. We used meta-analysis with a random-effects model to control for possible heterogeneity between contributing studies. Meta-analysis enables the use of information from studies that would not be interpretable by

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themselves or that cannot be assessed by simple pooling [3]. This study is limited by the retrospective nature of the data collected. Therefore, we were not able to analyse the data for dose relationship, which could support causality. Furthermore, permission to take samples for laboratory analysis in cases where symptoms had already ceased was seldom given, which led to a low number of patient samples tested in our study.

The illness that developed in the cases was characterized by symptoms and an onset time similar to those seen in toxin-mediated food poisonings [6, 7]. However, in one investigated outbreak, we did not detect staphylococcal enterotoxins (A, B, C, D, E) in the vomitus samples, even if the detection limit was as low as 1 ng/g in spiked samples. Nor did we detect traditional toxin-producing foodborne bacteria (*S. aureus*, *C. perfringens*, *B. cereus*) from the stool cultures collected from cases in three other outbreaks. Five of the studied outbreaks occurred during autumn when norovirus circulation is more intense, but only one of the nine tested cases was positive for norovirus by PCR detection, which may reflect prevalence of asymptomatic carriage in the general population [8]. Furthermore, the very sudden onset of symptoms after the meal does not support norovirus as a causal agent. The short incubation period, short duration and involved symptoms of this illness closely resemble the characteristics found in a series of school-meal-related outbreaks of gastrointestinal illness in the USA in 1997–1998 and 2003–2004 [9, 10]. The suspected vehicles of transmission in these outbreaks, tortilla breads, were thoroughly tested for a wide array of microbiological and chemical agents (bacterial, fungal and plant toxins, pesticides, food additives, biogenic amines, trace metals) without any conclusive findings.

Beetroot is usually consumed cooked or pickled in vinegar. Raw beetroot has a high content of nitrate (usually >1000 mg/kg) [11] but is considered to have several beneficial health effects, including physiological effects related to controlled nitrate supplementation [12] or to the content of betaine [13]. Beetroot also contains betalains, a group of antioxidant compounds [14] and polyphenols such as resveratrol, which may protect against a wide range of illnesses including cardiovascular disease [15]. In addition to being served in salad buffets as a promotion of healthy lunch menus, institutional kitchens serve raw domestic beetroot because of its low price and aesthetic property.

In our study, the quality of the beetroot served was poor according to the total bacterial counts, but no common foodborne pathogen could be identified. Moulds or mycotoxins were not tested from the beetroot samples. However, no visible moulds were reported by the farmers, production plants or kitchen staff. To our knowledge, no specific pathogen has been reported in conjunction with beetroot, unlike other vegetables used in a similar fashion, e.g. large outbreaks have occurred due to carrots contaminated with *Yersinia pseudotuberculosis* in Finland [16–19]. The investigation reports described long storage times for beetroot between peeling, grating and serving. The abrasive peeling used disrupts the outer layer of the beetroot, enhancing adhesion of bacteria, while the moist atmosphere in closed plastic bags enhances bacterial growth.

High yield of *P. fluorescens* was detected in several beetroot samples obtained from the institutional kitchens and originating from different producers and peelers. Refrigerated storage aims at preventing bacterial overgrowth and growth of pathogenic bacteria such as *Salmonella*, but refrigeration may enhance growth of psychrotrophic bacteria. In the dairy industry and aquaculture, *P. fluorescens* has been identified as spoilage bacteria [20–22], but its toxin-producing capability and adverse effect on human health has not been reported. Although traceback investigation revealed growth of *P. fluorescens* in the beetroot samples analysed in this study, there is hitherto no described biological mechanism bridging the causal gap between the exposure to raw beetroot and gastrointestinal illness. In order to identify new causal agents or toxins including possible toxins produced by *P. fluorescens*, tests for acute toxicity by cell cultures and animal models and further characterization by gas chromatography and mass spectrometry could be done [9].

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DECLARATION OF INTEREST

None.

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