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Yersinia pseudotuberculosis causing a large outbreak associated with carrots in Finland, 2006

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SUMMARY

A large outbreak of *Yersinia pseudotuberculosis* O:1 infection affected over 400 children from 23 schools and 5 day-care centres in two municipalities in southern Finland in August–September, 2006. A retrospective cohort study conducted in a large school centre showed that the outbreak was strongly associated with the consumption of grated carrots served at a school lunch. The risk of illness increased with the amount of carrots eaten. Poor quality carrots grown the previous year had been delivered to the school kitchens in the two municipalities affected. In the patients' samples and in the environmental samples collected from the carrot distributor's storage facility, identical serotypes and genotypes of *Y. pseudotuberculosis* were found, but the original source and the mechanism of the contamination of the carrots remained unclear. Outbreaks of *Y. pseudotuberculosis* linked to fresh produce have been detected repeatedly in Finland. To prevent future outbreaks, instructions in improved hygiene practices on the handling of raw carrots have been issued to farmers, vegetable-processing plants and institutional kitchens.

INTRODUCTION

Yersinia pseudotuberculosis is a zoonotic pathogen detected in various animal reservoirs around the world [1]. Human infections are characterized by fever and abdominal pain mimicking acute appendicitis [1, 2]. Extra-intestinal manifestations, such as reactive arthritis and erythema nodosum occur frequently [3, 4]. While reports of foodborne outbreaks caused by *Y. pseudotuberculosis* are rare worldwide, several outbreaks have been detected in Finland since the 1980s [3, 5].

On 29 August 2006, the National Public Health Institute (KTL) of Finland was notified by a hospital physician of 14 school-aged children with fever and acute abdominal pain in municipality A in southern Finland (35 434 inhabitants). Five of these children had undergone unnecessary appendectomy within a week. Over 30 other children with fever and abdominal pain had visited the municipal primary health-care

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centre. Furthermore, dozens of children from the neighbouring municipality B (32 305 inhabitants) had fallen ill with similar symptoms. Consequently, epidemiological, trace-back, environmental, and laboratory investigations were conducted. The aim was to determine the source and magnitude of the outbreak in order to prevent further outbreaks of *Y. pseudotuberculosis* infection.

SUBJECTS AND METHODS

Case-finding in the municipalities

A list of the patients who visited the health centre with abdominal pain or fever was maintained in municipalities A and B. In municipality A 276 persons of whom 200 (72 %) had abdominal pain combined with fever were listed. In municipality B, of 122 persons listed 77 (63 %) had abdominal pain combined with fever. Additionally, 29 persons attended hospital with symptoms including abdominal pain and fever.

Retrospective cohort study

We carried out a retrospective study in a cohort of 908 persons who attended the school lunch in a school in municipality A. The secondary school (855 pupils aged 12–19 years and 53 employees) was chosen for the study since 42/200 cases in municipality A originated from there and the pupils were old enough to complete the questionnaire reliably. The questionnaire took place on 14 September. Participants were asked about symptoms and consumption of school meals during a period from 15 to 25 August. They were encouraged to use a calendar and the school menu as a memory aid. In total, 845 persons returned the questionnaire, out of which 10 were unusable, leaving 835 (92 %) questionnaires for inclusion in the study.

A case was defined as a person with abdominal pain and fever ($\geqslant 38\ ^\circ C$) or erythema nodosum or laboratory-confirmed *Y. pseudotuberculosis* infection (stool culture between 20 August and 11 September 2006) who was present at the school during 15–25 August 2006.

Microbiological and trace-back investigation

Based on the standard procedures in foodborne outbreaks in Finland, stool specimens collected from five of the cases were analysed for *Salmonella*, *Shigella*, *Campylobacter* and *Yersinia* spp. as well as for *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens* by routine methods in two local clinical microbiology laboratories [6]. Thereafter, samples were cultivated for *Y. pseudotuberculosis* only. Altogether, 247 stool specimens taken between 15 August and 19 September were cultured on cefsulodin–irgasan–novobiocin (CIN) agar (Oxoid, Basingstoke, UK) for *Y. pseudotuberculosis* [6].

Trace-back investigations were conducted by the Finnish Food Safety Authority (Evira) in collaboration with the local environmental authorities and the food research laboratory TavastLab. Samples of food served on 21–31 August collected as a part of the internal quality control of the school kitchens as well as environmental samples from the storage facility of the vegetable distributor and the carrot farmer (Table 1) were tested [3, 7]. Surface swab samples were enriched in 225 ml peptone–mannitol–bile salt (PMB) broth (sorbitol of ISO 10273:2003 PSB broth replaced with 1 % of mannitol) at 4 °C for 5 days and 12 days. Food and environmental samples of 25 g were homogenized in 100 ml PMB, and 5 ml homogenate transferred to 95 ml fresh PMB and incubated at 4 °C for 7, 14, and 21 days. Alkali-treated samples (0·5 ml of the broth was mixed with 4·5 ml of 0·25 % KOH solution for 20 s) were streaked onto CIN agar and incubated at 30 °C for 48 h. *Y. pseudotuberculosis* isolates were identified using standard microbiological methods [6].

The laboratories sent *Y. pseudotuberculosis* isolates to KTL for verification and further analyses. In all, 83 human isolates and five isolates from the carrot distributor's storage facility were analysed by serotyping and pulsed-field gel electrophoresis (PFGE) as previously described [8]. Further subtyping of O:1 serotypes to O:1a and O:1b was obtained by PCR [9]. Presence of the virulence plasmid was detected by growth of red colonies on Congo Red magnesium oxalate agar [10].

Statistical analysis

Associations between food items and illness were assessed by univariate analysis using the χ^2 test. A backward stepwise binary regression model with log link was conducted for fresh produce that was significantly associated with the illness [11, 12]. Statistical analyses were conducted using Stata version 9.2 (Stata Corporation, College Station, TX, USA).

Table 1. *Food and environmental samples tested during* Yersinia pseudotuberculosis *outbreak in southern Finland, August–September, 2006*

Sample	Origin	*Y. pseudotuberculosis* O:1 detected/total samples studied
Grated carrots	School kitchens, municipalities A and B	0/6
Mixed vegetables	School kitchens, municipality A	0/13
	Vegetable distributor	0/3
Chopped and peeled carrots	Vegetable distributor	0/2
Carrot residues	Vegetable distributor	1/5
Rinsing water of carrots	Vegetable distributor	0/1
Surface sample from carrot processing equipment	Vegetable distributor	0/8
Surface sample from floor or wall	Vegetable distributor	3/10
	Carrot farmer	0/5

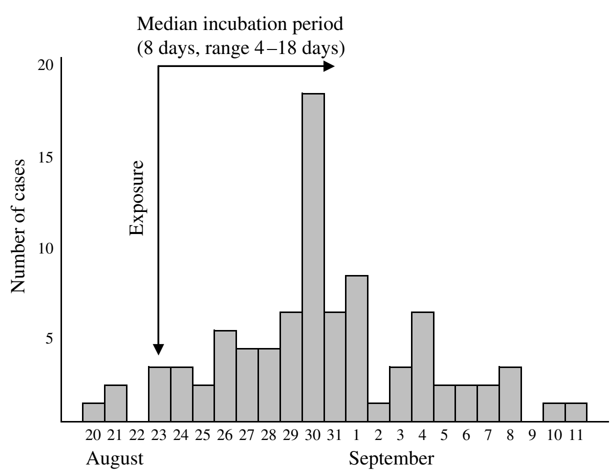

Fig. 1. Cases ($n = 104$), by date of onset of illness, among respondents of the questionnaire-based study, *Yersinia pseudotuberculosis* outbreak in southern Finland, August to September, 2006.

RESULTS

In total, 104/835 respondents (12%) met the case definition. The first case fell ill on 20 August. The incidence peaked on 30 August (Fig. 1). The cases ranged in age from 12 years to 60 years (median 15 years); 59% were females. Abdominal pain (101/104, 97% of cases) and fever (99/104, 95%) were the predominant symptoms. Other reported symptoms included back and joint pain (40/99, 40%; and 38/100, 38%, respectively), diarrhoea (20/98, 20%), erythema nodosum (14/96, 15%) and vomiting (14/98, 14%). Two (2%) cases were hospitalized, 35/102 cases (34%) visited a doctor, and 89/103 cases (86%) were absent from school due to the illness. The absences ranged from 1 to 15 days (median 4 days).

On univariate analysis, 22/25 food items served during the period 15–25 August were significantly associated with illness. The highest risk ratios (RR) were for fresh produce items (Table 2). In the multivariate binary regression model with backward selection, only grated carrots served on 23 August remained independently associated with illness at the 0·01 significance level [RR 2·3, 95% confidence interval (CI) 1·7–3·1]. A dose–response relation for eating grated carrots and risk of illness was detected. For those children who consumed plenty of grated carrots, compared to those who did not consume any, the RR was 9·9 (95% CI 5·1–19·1, $P < 0·001$) and for those who consumed some grated carrots the RR was 2·6 (CI 95% 1·3–5·5, $P = 0·009$). Carrots were served at the school on 15, 18 and 23 August.

The outbreak affected children from 23 schools and 5 day-care centres in the municipalities A and B. Trace-back investigation revealed that the school kitchens in towns A, B and two other municipalities shared a common vegetable distributor. The distributor had, however, supplied only the kitchens in municipalities A and B with the previous year's Finnish grated carrots. The grated carrots had not been washed in the school kitchen. The carrots could be traced back to one farm, where they had been stored for up to 6 months. The final batch of the carrots (3000 kg) had been stored at the vegetable distributor's storage facility for a further 4 months until the school opened in August. The carrots were of

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Table 2. *Consumption of fresh produce items on lunch at a school centre during* Yersinia pseudotuberculosis *outbreak in southern Finland, August–September, 2006*

Fresh produce item (date served)	Attack rate among exposed	Attack rate among non-exposed	Risk ratio (95% CI)	*P* value
Grated carrots (15 Aug.)	20·7 (79/381)	2·9 (7/242)	7·2 (3·4–15·3)	<0·001
Cucumber slices (16 Aug.)	19·6 (71/363)	4·5 (12/266)	4·3 (2·4–7·8)	<0·001
Iceberg lettuce (17 Aug.)	23·1 (69/299)	5·0 (15/303)	4·7 (2·7–8·0)	<0·001
Swede turnip/carrot slices (18 Aug.)	21·9 (47/215)	7·6 (30/395)	2·9 (1·9–4·4)	<0·001
Beetroot salad (21 Aug.)	18·7 (37/198)	8·9 (34/382)	2·1 (1·4–3·2)	0·001
Grated carrots (23 Aug.)	20·4 (69/339)	4·2 (11/261)	4·8 (2·6–8·9)	<0·001
Iceberg lettuce (24 Aug.)	18·0 (53/294)	8·5 (24/282)	2·1 (1·4–3·3)	0·001
Banana (25 Aug.)	17·4 (44/253)	9·2 (29/316)	1·9 (1·2–2·9)	0·004

CI, Confidence interval.

poor quality and a large proportion of the batch was destroyed. The rest were delivered to school kitchens in municipalities A and B.

All 83 patient isolates linked to the outbreak were serotyped as *Y. pseudotuberculosis* O:1. All of the first 50 isolates tested for the presence of a virulence plasmid carried it, indicating that the isolates were pathogenic. Fourteen randomly selected isolates were further studied by PFGE. They all had identical genetic type termed S12 (Fig. 2).

In the vegetable distributor's storage facility, *Y. pseudotuberculosis* serotype O:1 genotype S12 was detected in a carrot residue sample and in three surface samples (Table 1). The samples originated from the doorstep, and from the wall and floor of the storage facility where the previous year's carrots had been stored. In one sample, taken from the floor, *Y. pseudotuberculosis* serotype O:3 was detected. Both the human and environmental isolates of serotype O:1 belonged to subtype O:1b. All isolates contained the virulence plasmid. *Y. pseudotuberculosis* was not detected in the food samples and in the environmental samples originating from the carrot farmer.

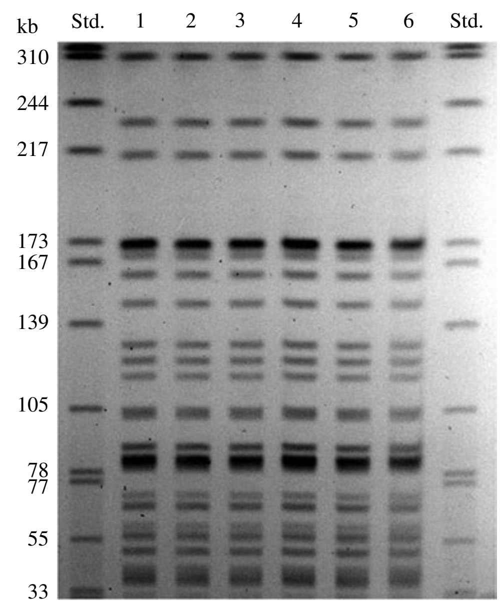

Fig. 2. PFGE patterns of *Spe*I-digested DNA of the isolates from *Y. pseudotuberculosis* outbreak, southern Finland, 2006. Lanes: Standard (Std.), *Salmonella* Braenderup digested with *Xba*I; lane 1, FE82881 human isolate (municipality B); lane 2, FE82911 human isolate (municipality A); lane 3, FE83039 carrot residues; lane 4, FE83041 surface sample (floor); lane 5, FE83042 surface sample (wall); lane 6, FE83043 surface sample (doorstep).

DISCUSSION

Over 400 persons in southern Finland fell ill during the large foodborne outbreak caused by *Y. pseudotuberculosis*. According to our study conducted in a large school centre, >10% of the participants had symptoms typical of *Y. pseudotuberculosis* infection leading to significant absence from school.

The school outbreak was strongly associated with exposure to grated carrots served on 23 August 2006. The risk of illness increased with the amount of carrots eaten; the risk was tenfold for those who consumed plenty of carrots compared to those who did not consume any. According to the study of Jalava *et al.* [3], the incubation period for *Y. pseudotuberculosis* infection ranges between 4 and 18 days (median 8 days). Sixteen cases that fell ill before 27 August were probably infected from the grated carrots served on 15 August. However, those carrots may have contained fewer bacteria, since the grated

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Table 3. Yersinia pseudotuberculosis *outbreaks in Finland 1997–2006*

Date	Number of educational institutes affected	Number of cases/laboratory-confirmed cases	Vehicle	Serotype	Reference
Aug. 1997	1	35/6	Unknown	O:3	KTL, unpublished data
Sept. 1998	1	60/11	Unknown	O:3	KTL, unpublished data
Oct.–Nov. 1998	2	/40	Iceberg lettuce	O:3	[8]
Oct.–Nov. 1999	3	31/25	Unknown	O:3	KTL, unpublished data
May–July 2001	2	/123	Unknown	O:3 and O:1	[2]
May 2003	36	111/79	Carrot	O:1	[3]
March 2004	5	58/7	Carrot	O:1	KTL, unpublished data
May–June 2006	13	42/17	Carrot	O:1	KTL, unpublished data
Aug.–Sept. 2006	28	427/119	Carrot	O:1	KTL, unpublished data

KTL, National Public Health Institute of Finland.

carrots on 23 August were the only exposure associated with infection as shown by the multivariate analysis. The incidence peak on 30 August (Fig. 1) is consistent with the result of the analytical study.

We used a retrospective cohort study, which is an appropriate study design for investigating foodborne outbreaks and allows direct calculation of risks. The school centre was selected for the study since many of the cases originated from there, the pupils were old enough to complete the questionnaire independently and all exposed in the school were able to be included in the study. We tried to minimize recall bias due to retrospective data collection by quick action; a team with questionnaire forms arrived at the school instead of mailing the forms. The team waited at the school until the forms were completed, which probably enhanced the participation rate.

The results of trace-back and environmental and laboratory investigations supported the result of the analytical study. The trace-back investigation revealed that poor-quality carrots grown the previous year had been delivered to the school kitchens in the two municipalities affected. In patients' samples and in environmental samples from the carrot distributor's storage facility, identical serotypes and genotypes of *Y. pseudotuberculosis* could be found. Unfortunately, most of the school food samples available for investigation dated from a period after 23 August. Moreover, the suspected carrot lot at the distributor's storage facility had already been destroyed by the time of sample collection.

Since 1997, repeated outbreaks caused by *Y. pseudotuberculosis* have been notified almost yearly in Finland and they have typically taken place at educational institutes (Table 3). *Y. pseudotuberculosis* outbreaks have also been reported in Russia and Japan [13–15], but surprisingly not in other Scandinavian countries. Fresh produce has increasingly been identified as a source of foodborne outbreaks [1] and in Finland, it is mostly raw carrots that have been linked to *Y. pseudotuberculosis* outbreaks. The bacterium serotype changed in 2001. In addition to the food item, the season, geography, animal reservoir or mere chance may have caused the change of the causative serotype. The genotype S12 detected in this study has been found in outbreaks since 2001, but the prevalence of different genotypes in humans or the environment is not known.

Finnish carrots are harvested in the autumn (September and October) and stored at 1–2 °C for up to 10 months on the farms. If the carrots have become contaminated by *Y. pseudotuberculosis* during cultivation or harvesting, the prolonged cold storage provides favourable circumstances for the bacteria to multiply. The carrots are cultivated in the open and irrigated mainly with surface water enabling contamination by many mammals and birds. A wildlife reservoir has been suspected and sought, but not yet discovered. Thus, the mechanism of contamination of the fresh produce remains in many respects unknown. Since *Y. pseudotuberculosis* infections are rare in the other Nordic countries, differences in the field-to-fork chain, in the manner of fresh produce consumption or in animal reservoirs in Finland compared to other Nordic countries might be present.

In Finland, carrots are one of the most popular items of fresh produce used in the institutional kitchens and in home kitchens [16]. Carrots are cultivated on about 700 farms and the yearly harvest is about 70 000 tons. In the spring of 2006, the Finnish

Food Safety Authority conducted a study to evaluate the contamination rate of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* in domestic carrots. No pathogenic *Yersinia* spp. were found in the 6-month survey [Evira, unpublished data] indicating that carrots are not regularly contaminated by *Y. pseudotuberculosis*. To prevent outbreaks in the future, farmers, vegetable-processing plants and institutional kitchens have been informed of the risk of *Y. pseudotuberculosis* arising from stored, domestic carrots. Furthermore, instructions to improve hygiene practices in storage and handling of raw carrots have been issued. This included the removal of carrots of poor quality, and re-washing of washed and peeled carrots before use. Recently, voluntary microbiological quality testing of raw carrots has been recommended to farms that store carrots until late spring.

Y. pseudotuberculosis is an emerging pathogen capable of causing widespread and severe foodborne outbreaks leading to hospitalization. Decreasing the possibility of contamination of fresh produce in cultivation, storage and processing are the cornerstones of prevention. To be able to focus prevention on the appropriate target, much remains to be learned about the epidemiology of the organism.

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DECLARATION OF INTEREST

None.

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