An Outbreak of Gastrointestinal Illness and Erythema Nodosum from Grated Carrots Contaminated with *Yersinia pseudotuberculosis*

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(See the editorial commentary by Frimodt-Møller and Hammerum, on pages 1191–3, and the article by Kieke et al., on pages 1200–8.)

**Background.** Outbreaks of *Yersinia pseudotuberculosis* infection have been epidemiologically linked to fresh produce, but the bacterium has not been recovered from the food items implicated. In May 2003, a cluster of gastrointestinal illness and erythema nodosum was detected among schoolchildren who had eaten lunches prepared by the same institutional kitchen.

**Methods.** We conducted a case-control study and trace-back, environmental, and laboratory investigations. Case patients had culture-confirmed *Y. pseudotuberculosis* O:1 infection, erythema nodosum, or reactive arthritis. Bacterial isolates from clinical and environmental samples were compared using pulsed-field gel electrophoresis (PFGE).

**Results.** Of 7392 persons at risk, 111 (1.5%) met the case definition; 76 case patients and 172 healthy control subjects were enrolled in the case-control study. Only raw grated carrots were significantly associated with illness in a logistic-regression model (multivariable odds ratio, 5.7 [95% confidence interval, 1.7–19.5]); a dose response was found for increasing amount of consumption. *Y. pseudotuberculosis* O:1 isolates from 39 stool specimens and from 5 (42%) of 12 soil samples that contained carrot residue and were obtained from peeling and washing equipment at the production farm were indistinguishable by PFGE.

**Conclusions.** Carrots contaminated early in the production process caused a large point-source outbreak. Our finding enable the development of evidence-based strategies to prevent outbreaks of this emerging foodborne pathogen.

*Yersinia pseudotuberculosis* infections occur primarily in the northern hemisphere. Clinical illness is characterized by fever and acute abdominal pain caused by mesenteric lymphadenitis, and it is often clinically indistinguishable from acute appendicitis [1, 2]. Postinfectious complications, such as reactive arthritis, are relatively common [3]. The incubation period for *Y. pseudotuberculosis* is unknown, but it is presumed to be 3–7 days on the basis of the organism’s similarity to *Y. enterocolitica* [4]. Evidence for foodborne transmission of this zoonotic pathogen of wild mammals (particularly rodents and lagomorphs) and birds is limited but has been reported through the ingestion of contaminated drinking water [5, 6], vegetable juice [7], and pasteurized milk [8] or through direct contact with infected animals [9].

Periodic outbreaks of *Y. pseudotuberculosis* infection, often in children in day-care centers and schools, continue to be a major public-health problem in many countries of temperate climates, such as the former Soviet Union, Japan, and those in northern Europe [1,
Cases of *Yersinia pseudotuberculosis* O:1 infection by date of onset of illness. Thirty-one case patients had culture confirmation, 9 case patients had culture confirmation and erythema nodosum, 35 case patients had erythema nodosum only, and 1 case patient had reactive arthritis.

Figure 1. Cases of *Yersinia pseudotuberculosis* O:1 infection by date of onset of illness. Thirty-one case patients had culture confirmation, 9 case patients had culture confirmation and erythema nodosum, 35 case patients had erythema nodosum only, and 1 case patient had reactive arthritis.

Specifi vehicles in these outbreaks, however, have rarely been implicated, and the sources of recurrent outbreaks have remained obscure. A recent epidemiologic investigation in Finland linked a nationwide outbreak of *Y. pseudotuberculosis* O:3 infection to domestically grown iceberg lettuce [12]. However, bacteria matching the outbreak subtype have not been previously recovered from epidemiologically implicated food items or an environmental reservoir.

On 23 May 2003, the Finnish National Public Health Institute (KTL) was notified about a cluster of *Y. pseudotuberculosis* infections among children attending school or day care in the town of Kotka (population, 55,000 persons) in southeast Finland. All ill persons had eaten lunches prepared by the same institutional kitchen. We conducted clinical, epidemiologic, trace-back, environmental, and laboratory investigations to determine the nature of illness, the magnitude and vehicle of the outbreak, and the source of contamination; we also identify opportunities for prevention.

SUBJECTS AND METHODS

Study population and case ascertainment. The source population included 7392 persons who attended or worked at a day-care center or school in which the lunches had been supplied by one institutional kitchen (kitchen A) from 26 April to 8 May 2003. There were 495 (7%) children 3–6 years old who attended day care (276 children <3 old years were excluded), 3574 (48%) schoolchildren 7–12 years old, 2649 (36%) schoolchildren 13–18 years old, and 674 (9%) day-care or school employees at risk.

A case patient was define as a person in the cohort with *Y. pseudotuberculosis* serotype O:1 isolated from stool culture or who had erythema nodosum or reactive arthritis diagnosed by a physician from 9 May to 3 June 2003. In Finland, clinical microbiology laboratories routinely screen stool specimens from patients with acute gastrointestinal illness for *Y. pseudotuberculosis*; confirmed infections are notifiable. Case patients were identify by active case finding at all 3 municipal health centers in Kotka and the single hospital and its clinical microbiology laboratory serving the health district. We also searched national laboratory notification to determine whether cases were occurring elsewhere in the country.

Case-control study. We conducted a nested case-control study among 76 case patients who had illness onset from 9 to 28 May and were identify by 31 May 2003. One case patient refused to participate.

The control subjects were selected as a stratified random sample of the noncases in the cohort, and they were matched to case patients by age-group frequency. We aimed to achieve
a matching ratio of at least 1:1 in each of the following age strata: day care, school grades, and adults. The sampling frame was created by merging municipal administrative, billing, and payroll records and represented a complete enumeration of the source population. Of 302 persons selected for the sample, 90 (30%) were considered to be unavailable after 4 unsuccessful contact attempts; 9 (3%) persons who did not speak Finnish or who were no longer employed were excluded. Of 203 persons contacted, 194 (96%) agreed to participate. Twenty-two (11%) were excluded because they reported fever and abdominal pain; 172 control subjects were enrolled.

Complete lunch menus were obtained from kitchen A. The study questionnaire included 27 separate food items and 3 drinks (milk, buttermilk, and water, which were served daily) consumed at the day-care centers and schools from 26 April to 8 May, a 2-week period before the first onset of illness. Trained study personnel obtained verbal informed consent from study subjects or their caretakers and conducted telephone interviews from 8 June to 3 July using a standard questionnaire. Food histories for the 8 weekdays when common source exposure was possible were self-reported for schoolchildren and adults; for children in day care, the personnel were interviewed. For persons <15 years old, information about symptoms of illness was provided by the caretaker. Clinical information about erythema nodosum and reactive arthritis was collected by chart review.

Stool samples obtained from 52 (68%) of 76 case patients enrolled in the case-control study were cultured for Y. pseudotuberculosis at the local clinical microbiology laboratory using routine methods. Bacterial isolates were sent to KTL for serotyping and pulsed-field gel electrophoresis (PFGE) analysis.

Trace-back and environmental investigation. Kitchen A prepares ~7000 meals daily for all 25 schools and 11 (32%) of 34 day-care centers in Kotka. No food items from 26 April to 8 May were available for culture. Retail invoices and shipping records were obtained for all food items served; all domestic fresh produce on the menu was traced back to its producer.

On 15 July, a total of 26 environmental samples were collected from the production farm (producer A) where the carrots implicated by the case-control study had originated. Twelve soil samples containing carrot residue were taken from the initial areas of carrot washing and peeling equipment, and 14 surface samples were taken from the end of the production line. Environmental samples were enriched in phosphate-mannitol-peptone broth and in phosphate-mannitol-bile broth at 4°C and were subcultured weekly for 3 weeks after KOH (0.25% in saline) treatment onto cefsulodin-irgasan-novobiocin agar and MacConkey agar and incubated for 48 h at 30°C. To estimate the level of contamination, 2 of the samples with carrot residue were inoculated with cfu of a non–outbreak-related Y. pseudotuberculosis O:1 strain and incubated as were the primary soil samples.

Isolate subtyping. Y. pseudotuberculosis isolates from clinical and environmental samples were verified and serotyped as described elsewhere [10, 12, 13]. PFGE was performed and analyzed for banding patterns as described elsewhere [10, 14, 15] using NotI and SpeI (MBI Fermentas) digestion with the following modifications: bacterial cells were suspended on 2 mL of CSB (100 mmol/L Tris and 100 mmol/L EDTA [pH 8.0]) to an OD_{260} of 0.36–0.42.

Statistical analysis. Data were analyzed using Epi Info (version 6.04; Centers for Disease Control and Prevention),

### Table 1. Characteristics of 76 case patients enrolled in the case-control study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case patients with the characteristic, no./total no. (%)</th>
<th>Culture-confirmed (n = 40)*</th>
<th>Erythema nodosum (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt;15 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td></td>
<td>20/40 (50)</td>
<td>20/36 (56)</td>
</tr>
<tr>
<td>Fever (temperature &gt;38°C)</td>
<td></td>
<td>37/40 (93)</td>
<td>33/36 (92)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td></td>
<td>33/40 (83)</td>
<td>32/36 (90)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td></td>
<td>8/39 (21)</td>
<td>10/36 (28)</td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td>9/39 (23)</td>
<td>4/36 (11)</td>
</tr>
<tr>
<td>Joint or back pain</td>
<td></td>
<td>20/40 (50)</td>
<td>19/36 (53)</td>
</tr>
<tr>
<td>Hospitalized</td>
<td></td>
<td>7/40 (18)</td>
<td>2/36 (6)</td>
</tr>
<tr>
<td>Duration of stay, median, days</td>
<td></td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Underwent appendectomy</td>
<td></td>
<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Length of illnessb</td>
<td></td>
<td>17 (5–36)</td>
<td>18 (1–37)</td>
</tr>
</tbody>
</table>

* Nine case patients with culture-confirmed infection also had erythema nodosum.

b Six case patients still had symptoms at the time of the interview. The duration of illness was recorded as lasting until the interview date.
SPSS (version 11.2; SPSS), and Stata (version 8 for survey data; StataCorp) software. We used the Mantel-Haenszel (M-H) method to calculate summary odds ratios (ORs) adjusted for age group, the frequency-matching variable. Food items significantly associated with illness in the primary analysis and age group (day care, school grades, and adults) were included in an unconditional logistic-regression model. Forward selection was used to identify independent risk factors. Logistic regression was also used for dose-response analysis to obtain adjusted ORs and 95% confidence intervals (CIs). The total number of ill individuals in the source population with t-distributed 95% CIs was estimated by using the distribution of 22 persons with fever and abdominal pain in the stratified random sample, the selection probability, and the number of healthy control subjects in each age stratum.

RESULTS

Description of the outbreak. We identified a total of 111 (1.5%) persons who met the case definition in the source population: 58 (52%) had *Y. pseudotuberculosis* O:1 isolated from stool, 52 (47%) had erythema nodosum only, and 1 (0.9%) had reactive arthritis. Dates of onset of illness were available for 76 case patients; the incidence of infections peaked during the second week of May (figure 1). The age-specific attack rates were 3.6% (18/495) among children 3–6 years old, 1.7% (62/3574) among schoolchildren 7–12 years old, 0.9% (23/2649) among schoolchildren 13–18 years old, and 1.2% (8/674) among school and day-care staff.

Clinical information was collected for 76 case patients (table 1). They ranged in age from 4 to 52 years (median, 10 years); 87% were <15 years old, and 53% were male. *Y. pseudotuberculosis* O:1 was isolated from 40 (77%) of 52 stool samples obtained. Thirty-nine isolates had indistinguishable PFGE patterns by both SpeI and NotI restriction enzymes; 1 isolate differed by 1 band (figure 2). Fever and abdominal pain were the predominant symptoms; only 24% of case patients had diarrhea. Case patients with a positive stool culture were as likely to report gastrointestinal symptoms as were those with ery-
Table 2. Reported consumption of fresh produce items on the lunch menu in day-care centers and schools, Kotka, Finland, from 28 April and 9 May 2003 among case patients with Yersinia pseudotuberculosis O:1 infection and among control subjects.

<table>
<thead>
<tr>
<th>Fresh produce item (date served)</th>
<th>Case patients eating the item, no./total no. (%)</th>
<th>Control subjects eating the item, no./total no. (%)</th>
<th>M-H odds ratioa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh salad (29 April)</td>
<td>53/68 (78)</td>
<td>109/151 (72)</td>
<td>1.3 (0.7–2.7)</td>
</tr>
<tr>
<td>Grated carrots (5 May)</td>
<td>65/69 (94)</td>
<td>118/157 (75)</td>
<td>5.0 (1.8–17.1)</td>
</tr>
<tr>
<td>Summer salad (6 May)</td>
<td>52/64 (81)</td>
<td>94/142 (66)</td>
<td>2.3 (1.1–4.9)</td>
</tr>
<tr>
<td>Green salad (7 May)</td>
<td>54/63 (86)</td>
<td>102/143 (71)</td>
<td>2.5 (1.1–5.9)</td>
</tr>
<tr>
<td>Frozen berries (8 May)</td>
<td>48/63 (76)</td>
<td>102/156 (65)</td>
<td>1.6 (0.8–3.3)</td>
</tr>
</tbody>
</table>

*NOTE.* Only persons for whom data on consumption of each fresh produce item are available are included. CI, confidence interval.

* M-H method was used to calculate summary odds ratios adjusted for age group.

The median incubation period to the onset of first symptom of Y. pseudotuberculosis infection was 8 days (range, 4–25 days) (figure 1). The median incubation period to the onset of acute abdominal symptoms (abdominal pain, diarrhea, or vomiting) was also 8 days (range, 4–18 days); that to the onset of erythema nodosum was 19 days (range, 6–29 days) (figure 3). On the basis of the prevalence of fever and abdominal pain in the random sample, the total number of ill persons in the cohort was estimated to be 558 (95% CI, 274–842).

Trace-back and environmental investigation. The carrots implicated in the case-control study were traced back to the production farm. There were no domestic animals on the farm or near the unfenced fields. No animal manure was used as fertilizer, nor were the carrots irrigated. The carrots delivered to kitchen A were from the crop of fall 2002 and had been stored in open containers in an unenclosed barn over the winter, accessible to storage rodents. Producer A washed, peeled, and packed the carrots in 5- and 10-kg bags. Distributor A in Kotka bought 325 kg of carrots on 30 April 2003 and delivered 280 kg of these on 2 May to kitchen A, where raw carrots were stored refrigerated and were grated for consumption on 5 May without additional washing or cleaning.

No implicated carrots delivered to kitchen A were available for culture. However, Y. pseudotuberculosis O:1 with a PFGE...
pattern indistinguishable from that of the outbreak subtype was isolated from 5 (42%) of 12 soil samples containing carrot residue using both SpeI and NotI restriction enzymes; 1 Y. pseudotuberculosis serotype O:1 strain with a different PFGE pattern was also isolated (figure 2). All 14 surface samples tested negative by culture. In the inoculum experiment, the estimated level of Y. pseudotuberculosis contamination in the samples from the production line was $1 \times 10^3$ cfu.

Distributor A also delivered carrots from the 30 April batch to 5 other commercial establishments in the Kotka area. One of these was associated with 2 cases of illness caused by the outbreak subtype (figure 2). Active national surveillance identified a cluster of 12 children with Y. pseudotuberculosis O:1 infection in the city of Tampere with onset of illness from 1 May to 21 May 2003. All 8 isolates available had PFGE patterns indistinguishable from that of the outbreak subtype (figure 2). During April and May, producer A had delivered carrots to schools in Tampere; he had no other customers.

**DISCUSSION**

This large point-source outbreak was caused by raw carrots contaminated by Y. pseudotuberculosis at the farm of origin. To our knowledge, our investigation is the first to demonstrate the isolation of the Y. pseudotuberculosis outbreak subtype from environmental samples obtained from the production line of an epidemiologically implicated food vehicle of the outbreak. A dose-response relation was observed for eating increasing amounts of grated carrots and the likelihood of illness. Another contemporary cluster of infections in a different geographical area was caused by an identical Y. pseudotuberculosis subtype and was also linked to carrots from the same producer. Our investigation aids the understanding of the phenomenon of recurrent Y. pseudotuberculosis outbreaks affecting children at day-care centers and schools [10] and enables the development of evidence-based public-health strategies to prevent these outbreaks.

This was among the largest foodborne outbreaks detected in Finland since surveillance began in 1975. The identifiable case patients accounted only for approximately one-fifth of the estimated 558 persons with gastrointestinal illness among those eating meals prepared by a large institutional kitchen. The clinical diagnosis of Y. pseudotuberculosis infection is difficult [16]. Many patients had only fever and abdominal pain or mild symptoms, and stool cultures were not always requested. Because only one-quarter of the case patients had diarrhea and routine cultures may not detect Yersinia species, our finding...
underscore the importance of testing stool specimens for these organisms during outbreaks where the predominant symptoms are fever and abdominal pain [12].

The point-source nature of exposure enabled us, for the first time, to accurately determine the incubation period for Y. pseudotuberculosis infection and, thus, further contribute to the clinical evidence base of this enigmatic organism. The incubation period to the onset of acute gastrointestinal symptoms was 4–18 days, which is substantially longer than the previously quoted 3–7 days that was extrapolated from studies of Y. enterocolitica outbreaks [4]. The clinical illness was severe and long-lasting; erythema nodosum developed in more than one-half of case patients as a postinfectious complication [3, 17, 18].

Fresh produce is increasingly recognized as a source of foodborne illness and outbreaks worldwide [19–22]. During the past few decades, the number of foodborne outbreaks associated with fresh produce has increased; they also account for an increasing proportion of reported outbreaks. Generally, outbreaks associated with fresh produce are often larger than those caused by foods of animal origin, and they may involve many states or countries [19, 20, 23, 24]. Recurring Y. pseudotuberculosis outbreaks affecting children in day-care centers and schools is a well-known clinical phenomenon in the former Soviet Union [25]. These outbreaks have often been thought, on the basis of anecdotal information, to be associated with fresh produce, but no evidence from peer-reviewed literature is available. Lack of regulations for traceability often makes the tracing back of implicated fresh produce difficult [12, 20]. Because accurate billing and shipping records were available at the institutional kitchen, we were able to trace back the carrots to a single domestic farm.

Raw carrots are an uncommon vehicle of foodborne outbreaks: they have been implicated in only 2 outbreaks in England and Wales during 1992–2005 (I. Gillespie, Health Protection Agency, personal communication) and in 2 outbreaks in the United States during 1973–1997 [20]. In our investigation, no carrots from the implicated batch were available for culture. Because of a short shelf life, the suspected produce item is usually long gone by the time the outbreak is detected. Y. pseudotuberculosis has rarely been isolated from food items [26, 27]. Nevertheless, one-half of the samples from the carrot washing and peeling equipment grew the outbreak subtype ≥2 months after the implicated batch was processed, and the results of the inoculation experiment suggested relatively high levels of contamination.

The exact mechanism of the contamination of carrots in the farm remains unknown. Rodents and/or other wildlife reservoir animals had access to the carrots stored in open containers in a barn during winter. A combination of direct contact with wildlife feces during storage and cross-contamination of the equipment during washing and peeling are the most likely contributing factors. Storage of carrots at low temperatures in the institutional kitchen for 5 days before serving may have further favored the growth of Y. pseudotuberculosis [10]. The carrots were also not washed in the kitchen before they were shredded and served. However, in addition to surface contamination, certain pathogens have been found to enter into vegetables and therefore cannot be completely washed off [28].

Y. pseudotuberculosis has been presumed to be a foodborne pathogen by virtue of its similarity to Y. enterocolitica, but documentation of transmission through food has only recently been obtained [12, 29]. Our investigation has demonstrated that Y. pseudotuberculosis is an emerging foodborne pathogen that can cause large outbreaks associated with severe illness and frequent postinfectious complications. Our finding enables the development of evidence-based strategies to prevent the contamination of carrots and other fresh produce at the production farm and have implications for public-health policy: practices that may increase the risk of contamination during storage and the production process, such as access by wild animals to storage facilities and inadequate cleaning of processing equipment, should be corrected during routine inspections of production facilities. To prevent outbreaks, regulations addressing the production, storage, and shipping conditions for fresh produce are needed in the European Union [30].

Future research should define the wild animal reservoirs of this infection and identify the risk factors associated with sporadic illness. Experiments should be designed to study the behavior of Y. pseudotuberculosis in carrots during storage and the potential for internal contamination. Most reported Y. pseudotuberculosis infections in Finland are not part of recognized outbreaks. Some of these infections, however, may be due to carrots or other fresh produce contaminated during growing, production, and processing.

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References