A Widespread Outbreak of *Yersinia pseudotuberculosis* O:3 Infection from Iceberg Lettuce

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(See the editorial commentary by Tauxe, on pages 761-3.)

Background. The vehicles and sources of *Yersinia pseudotuberculosis* infection are unknown. In Finland, clinical microbiology laboratories routinely report *Y. pseudotuberculosis* isolations and submit isolates for serotype analysis. In October 1998, the number of serotype O:3 infections increased markedly.

Methods. Case patients with culture-confirmed *Y. pseudotuberculosis* O:3 infection were identified by use of laboratory-based surveillance. We conducted a population-based case-control study. Healthy community control subjects were matched by age, sex, and postal code. Isolates were subtyped by pulsed-field gel electrophoresis (PFGE).

Results. Nationwide, 47 case patients were identified (age range, 2–77 years; median, 19 years). One patient with bacteremia died; 5 underwent appendectomies. We enrolled 38 case patients and 76 control subjects in the case-control study. Seventy-one percent of case patients and 42% of control subjects reported having eaten iceberg lettuce (matched odds ratio, 3.8; 95% confidence interval, 1.3–9.4); a dose-response relationship was found for increasing frequency of consumption. Of the 27 isolates obtained from case patients and tested in the analysis, all had indistinguishable PFGE patterns. Four lunch cafeterias that had served iceberg lettuce were associated with clusters of case patients. The lettuce was traced back to originating farms.

Conclusions. Iceberg lettuce was implicated as the vehicle of a widespread foodborne *Y. pseudotuberculosis* outbreak. Ongoing laboratory-based surveillance and serotype analysis were essential in the rapid detection of infection. Cases of yersiniosis, which appear to be sporadic, may be part of unrecognized outbreaks caused by contaminated fresh produce.

Yersinia pseudotuberculosis infections are characterized by fever and abdominal pain due to mesenteric lymphadenitis, which, in the clinical setting, is frequently indistinguishable from acute appendicitis [1, 2]. Occasionally, in individual cases, transmission has been reported to have occurred by contact with infected animals [3] or by drinking contaminated water [4]. Reports of community outbreaks are rare. They have mainly occurred in Finland [1, 2], Japan [5, 6], and the former Soviet Union; 1 outbreak in Canada has also

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been reported [7]. However, previous investigations have not implicated specific vehicles of the outbreaks. In contrast to what is known about the epidemiology of *Y. enterocolitica* [8–10], the sources and vehicles of *Y. pseudotuberculosis* infection are obscure, and foodborne transmission has been poorly documented.

In October 1998, 2 clinical microbiology laboratories in southern Finland noted a marked increase in *Y. pseudotuberculosis* isolations. Laboratories in other regions also reported infections. All isolates were serotype O:3. The National Public Health Institute (KTL) initiated epidemiologic and laboratory investigations to determine the extent and source of the geographically widespread outbreak. Subsequently, we conducted traceback and prospective environmental investigations.

SUBJECTS, MATERIALS, AND METHODS

Population-based surveillance. In Finland, clinical microbiology laboratories routinely screen stool specimens from patients with acute gastroenteritis for *Yer*-

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sinia species, in addition to *Salmonella, Shigella*, and *Campy-lobacter* species, in accordance with national guidelines. Reporting of *Y. pseudotuberculosis* isolations to the National Infectious Disease Registry's database is mandatory. Most laboratories notify electronically; they also forward bacterial isolates to KTL for serotype analysis.

A case was defined as an illness in which *Y. pseudotuberculosis* serotype O:3 was isolated by culturing of stool or blood specimens from 15 October to 6 November 1998. Culture-confirmed cases were identified by searching routine notifications and by active case finding at all clinical microbiology laboratories in the country's 22 health districts. In addition, data on cases of pseudoappendicitis defined on the basis of surgical diagnosis and cases diagnosed by serologic testing only (elevated level of specific IgM antibodies against *Y. pseudotuberculosis* O:3, by EIA) were collected on a line list.

Laboratory methods. Local clinical microbiology laboratories isolated *Y. pseudotuberculosis* by use of routine methods [11]. At KTL, *Y. pseudotuberculosis* isolates were serotyped by slide agglutination (Denka Seiken) and were compared by pulsed-field gel electrophoresis (PFGE), by use of previously proposed criteria [12]. Preparation of plugs and digestion of chromosomal DNA with *Not*I and *SpeI* restriction enzymes were performed as described elsewhere [13]. Additional overnight incubation with lysozyme and RNase, before incubation with proteinase K, was required. PFGE was performed by use of the CHEF Mapper system (Bio-Rad Laboratories).

Case-control study. To determine factors associated with acquiring yersiniosis, we conducted a population-based case-control study. The 38 culture-confirmed case patients were residents of 2 southern health districts (aggregate 1998 population, 1.8 million), where 85% of cases were identified. One patient refused to participate, and another patient, who died, was excluded from the study.

Control subjects were identified from the general population. For each case patient, a list of persons who matched the patient, according to year of birth, sex, and postal code of residency, was generated from the National Population Information System. From each list, we enrolled 2 randomly selected persons as control subjects. Persons with abdominal pain, diarrhea, or vomiting and patients who travelled abroad during the month before the interview were excluded. Of 82 eligible persons invited to participate, 76 (93%) agreed to participate.

Trained personnel obtained informed consent from the study subjects or their caregivers and conducted telephone interviews from 2 to 17 December 1998 using a standard questionnaire. For persons <15 years old, the caregiver was interviewed. Participants were asked about illness; consumption of fresh produce, meat products, and untreated water; shopping locations; and meals eaten outside the home. For case patients, the questions referred to the 2 weeks before onset of illness, and, for control subjects, the questions referred to the 2 weeks before the interview. Participants were asked to use a calendar as a memory aid to recall exposures. Because the present study was conducted as part of the investigation and control of an acute public health problem, no ethics committee approval was required.

Trace-back and environmental investigations. Four lunch cafeterias were associated with distinct clusters of case patients. We reviewed cafeteria menus and requested retail invoices for iceberg lettuce purchased, because it had been implicated by the case-control study. Bills of lading and shipping records were obtained from 3 local distributors that had sold iceberg lettuce to these cafeterias and from the 1 shipping company that had supplied all local distributors.

In May 1999, 4 farms implicated by the trace-back investigation were inspected for sanitary conditions and water quality. The farmers were interviewed about lettuce growing, irrigation, and harvesting practices; handling practices during distribution were also inspected. To study the natural ecology of *Y. pseudotuberculosis* during the lettuce growing season, we conducted prospective environmental sampling in the 4 farms from May to November 1999 and again from June to October 2000.

A total of 287 samples were collected from lettuce, surface soil, animal feces found in the fields, and the irrigation water system (51 from soil, 21 from sludge, 22 from feces, 39 from water, 128 from lettuce, 4 from compost, and 22 from water pipes). Samples were transferred to 90 mL of PBS with 1.0% mannitol and 0.15% bile salts (PMB). Water samples were filtered through a 0.45- μ m membrane and placed in 90 mL of PMB. Samples were cultured for *Y. pseudotuberculosis*, as described elsewhere [14]; 100 μ L of PMB homogenate was directly plated onto selective cefsulodin-irgasan-novobiocin agar plates (Yersinia Selective Agar Base; Oxoid). After cold enrichment, 1–4 colonies suspected to contain *Y. pseudotuberculosis* were plated onto blood agar and were tested for urea hydrolysis. Urease-positive isolates were identified by use of the API 20E system (BioMérieux).

Statistical analysis. Mantel-Haenszel matched odds ratios (MORs) and 95% exact confidence intervals (CIs; for categorical variables), Kruskal-Wallis test (for continuous variables), and the χ^2 test (for trend) were calculated by use of Epi Info software (version 6.04; Centers for Disease Control and Prevention).

RESULTS

Description of the outbreak. From 15 October to 6 November 1998, 47 cases of culture-confirmed *Y. pseudotuberculosis* O:3 infection were identified nationwide. The incidence of infection peaked during the last week of October (figure 1). The case patients ranged in age from 2 to 77 years (median, 19 years); 53% were female, and 40% were <15 years old. Fever and abdominal pain were the predominant symptoms; less than

one-half had diarrhea (table 1). None had a chronic illness or immunodeficiency. Sixteen case patients (34%) were hospitalized, and 5 of them underwent appendectomies before diagnosis of yersiniosis had been made. *Y. pseudotuberculosis* O:3 was cultured from the blood specimens of 2 patients. A 77year-old previously healthy woman with bacteremic infection died within 24 h of admission to the hospital.

Residents of 2 health districts in southern Finland (A and B) accounted for 40 cases (85%). In these districts, the attack rate per 100,000 persons was 2.2 overall and 5.1 among children <15 years old. All 27 isolates from case patients that were available for testing (14 from district A and 13 from district B) had indistinguishable PFGE patterns by digestion with 2 restriction enzymes and were different from isolates of unrelated, sporadic cases (figure 2). During 1998, 6 additional Y. pseudotuberculosis O:3 isolates submitted to KTL as a result of routine surveillance were subtyped by PFGE. Three different patterns were identified. One isolate from July 1998 was similar to the outbreak strain. In a follow-up clinical evaluation that included 33 case patients, 10 (30%) reported joint symptoms, and 4 (12%) had reactive arthritis, defined as a new joint symptom, including arthralgia, redness, and/or swelling in ≥1 joints, within 1 month of Yersinia infection [15].

Case-control study. We enrolled 38 case patients (table 1) and 76 control subjects in the case-control study. In matched analysis, only 1 of the food items that participants were asked about was significantly associated with illness: 71% of case patients and 43% of control subjects reported having eaten iceberg lettuce (MOR, 3.8; 95% CI, 1.3–9.4; table 2). Carrots were consumed by 69% of case patients, and the point estimate for MOR was 2.3. However, the CI included 1, and 18 case patients (75%) who reported having eaten carrots also reported having eaten iceberg lettuce. After stratification by consumption of iceberg lettuce, the adjusted MOR for carrots was 1.5 (95% CI,

0.5–6.5). Consumption of other types of lettuce, fresh produce, water, milk, pork, or beef was not associated with illness.

Of the different types of lettuce consumed, only iceberg lettuce was eaten more often by case patients than by control subjects during the 2-week interview period (median, 5 times vs. 2 times; P = .002). The odds of illness increased with the number of times iceberg lettuce was consumed, suggesting a dose-response relationship (P < .001, test for trend; table 3).

Trace-back investigation. We identified clusters of case patients and patients who underwent appendectomies among children in 2 primary schools in health district A and among employees of 2 manufacturing plants in health district B. Eight culture-confirmed case patients were associated with these clusters and were used as the basis for the trace-back investigation. In addition, 6 persons in these clusters had serologic evidence of recent Y. pseudotuberculosis O:3 infection, but these findings were not confirmed by culture; 9 persons underwent appendectomy, but only 2 of them underwent diagnostic testing (figure 3). Iceberg lettuce was served at all 4 lunch cafeterias during the case patients' incubation periods (illness onset for cultureconfirmed cases occurred from 15 to 26 October). At each facility, the trace-back investigation narrowed the likely source of infection to a single batch of purchased lettuce. In each district, a local fresh-produce distributor (A and C) delivered the iceberg lettuce to 2 cafeterias; distributor C received the lettuce from intermediate distributor B. Both distributors A and B obtained lettuce from only 1 fresh-produce shipping company. Lettuce shipped by shipper A during 4-8 October was distributed to all 4 cafeterias, and this could explain all infections associated with the clusters.

Shipper A obtained lettuce from farms in the southwest archipelago region, which accounts for \sim 15% of the 4000 tons of iceberg lettuce produced in Finland annually. At the farms, shipper A packed the lettuce heads into 5-kg cardboard boxes

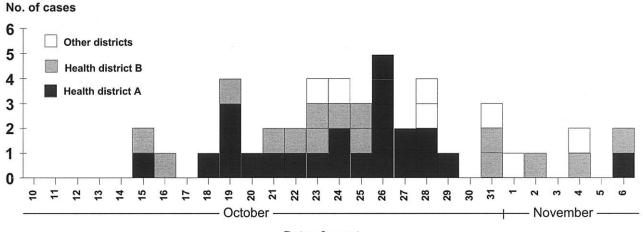




Figure 1. Culture-confirmed cases of Yersinia pseudotuberculosis 0:3 infection, according to the date of onset of illness

 Table 1.
 Characteristics of case patients with culture-confirmed Yersinia pseudotuberculosis 0:3 infection and of those enrolled in the case-control study.

	Case patients		
Characteristic	With culture-confirmed infection (n = 47)	Enrolled in case-control study (n = 38)	
Age, median (range), years	19 (2–77)	16.5 (2–51)	
<15 years old	19/47 (40)	17/38 (45)	
Female	25/47 (53)	21/38 (55)	
Symptoms			
Diarrhea	20/44 (45)	18/37 (49)	
Abdominal pain	39/44 (89)	33/36 (92)	
Fever (temperature >38°C)	30/44 (68)	24/37 (65)	
Hospitalized	16/47 (34)	14/37 (38)	
Duration of stay, median, days	3	3	
Underwent appendectomy	5/47 (11)	5/38 (13)	
Extraintestinal manifestations			
Joint symptoms	NA	10/33 (30)	
Reactive arthritis	NA	4/33 (12)	

NOTE. Data are no. of case patients with characteristic/no. of case patients for whom data on characteristics are available (%), unless otherwise noted. NA, not applicable.

and loaded these directly into trucks. The lettuce heads were not rinsed or washed before shipping. There was no intermediary storage, and the lettuce was not handled or unpacked until it was delivered to the point of service. During 4–8 October, wholesale distributors A and B sold 2550 kg of iceberg lettuce to local and national distributors, brokers, and retail chains, mainly in southern Finland. These were among the last domestically produced shipments before the distributors began importing iceberg lettuce from abroad in mid-October.

On the basis of bills of lading, 4 farms were possible sources of the iceberg lettuce sold to the cafeterias associated with clusters of case patients. Each of these farmers sold their entire crop to shipper A. Iceberg lettuce originating from farm A on 5 October was delivered to all 4 cafeterias during 7–9 October and was served on multiple days during the following week (12–16 October). However, because of incomplete documentation, we could not rule out the possibility that lettuce from the 3 other farms could also have ended up in some or all of the cafeterias. Since repeated harvests of iceberg lettuce continued until 25 October 1998 and since the last onset of cultureconfirmed illness occurred on 6 November 1998, infections outside these clusters may have been due to later-harvested lettuce lots.

Environmental investigation. No implicated iceberg lettuce was available for culture by the time the trace-back investigation had been completed in December 1998 and snow and ground frost made environmental sampling impossible. The 4 farms were therefore inspected in May 1999. In all farms, 2 or 3 crops of iceberg lettuce were grown in open fields, alternating with Chinese cabbage, beginning in early May. The last crops of iceberg lettuce were harvested on 25 October 1998. No animal manure was used as fertilizer, and there were no domestic livestock near the fields. Untreated water was used for spray irrigation of the fields. Farm A and another farm obtained water through a ditch from a nearby lake, whereas, in 2 other farms, water came from man-made ponds supplied by rainwater, ditches, and surface run-off. Fields were unfenced, and wildlife had free access to irrigation water sources and

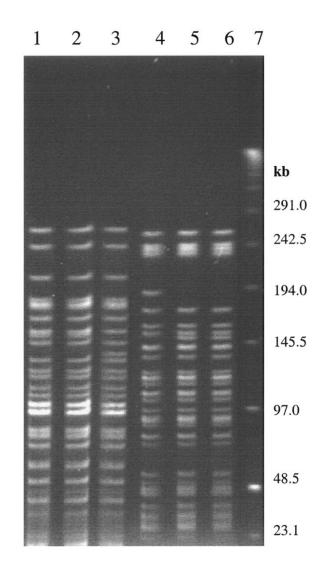


Figure 2. Pulsed-field gel electrophoresis patterns of *Yersinia pseudotuberculosis* 0:3 isolates. Chromosomal DNA was digested with 2 enzymes (*Spel* and *Notl*). *Lane 1*, An isolate from a patient in health district B, digested with *Spel* enzyme; *lane 2*, an isolate from a patient in health district A (*Spel*); *lanes 3* and 4, an unrelated isolate from a sporadic case in September 1997, digested with *Spel* and *Notl* enzymes, respectively; *lanes 5* and 6, the same isolates as shown in lanes 1 and 2, digested with *Notl*; *lane 7*, standard, low-range pulsed-field gel marker.

	No. of exposed subjects/ total no. of subjects (%)			
Food item	Case patients $(n = 38)$	Control subjects $(n = 76)$	MOR (95% CI)	Ρ
Fresh produce				
Iceberg lettuce	27/38 (71)	30/69 (44)	3.8 (1.3–9.4)	.01
Carrots	24/35 (69)	36/74 (49)	2.2 (0.9–6.5)	.08
White cabbage	5/34 (15)	8/71 (11)	1.2 (0.3–5.0)	.1
Chinese cabbage	20/35 (57)	33/69 (48)	1.3 (0.6–3.3)	.6
Leaf lettuce (individually grown)	18/36 (50)	38/72 (53)	0.9 (0.3–2.3)	.9
Green leaf lettuce	6/37 (16)	16/71 (23)	0.6 (0.2–2.1)	.6
Onions	14/38 (37)	27/74 (28)	1.0 (0.4–2.4)	.9
Leeks	5/36 (14)	12/74 (16)	0.8 (0.2–3.2)	.9
Other				
Premade salads	11/33 (33)	21/74 (28)	1.5 (0.5–4.2)	.6
Ground beef	27/32 (84)	59/70 (84)	0.9 (0.3–3.8)	.8
Pork				
Any	34/37 (92)	71/76 (93)	0.8 (0.2–5.4)	.9
Filet	14/32 (44)	22/68 (32)	1.2 (0.4–3.9)	.8
Chops	8/32 (25)	14/70 (20)	1.2 (0.4–3.9)	.9
Cold cuts (ham)	30/38 (79)	55/72 (76)	1.2 (0.4–3.5)	.1

Table 2.	Reported consumption of selected food items, among case patients with Yersinia			
pseudotuberculosis 0:3 infection and among control subjects matched by age, sex, and postal				
code.				

NOTE. Only persons for whom data on consumption of each food item are available are included. Cl, confidence interval; MOR, matched odds ratio.

fields. We found no evidence of the presence of unusual numbers of small rodents or lagomorphs on the farms. However, an ecologically distinct feature of the southwest archipelago is a large population (>10,000 animals) of nonnative roe deer *(Capreolus capreolus)* that were introduced to the islands during the 1960s. Large quantities of roe deer feces were found all over the lettuce fields and around all irrigation water sources.

Of the 287 samples obtained, 72 (25%) yielded bacteria of *Yersinia* species. *Y. pseudotuberculosis* was recovered from 1 soil and from 1 irrigation water sample, in November 1999, and from 2 iceberg lettuce samples in October 2000. All positive samples were obtained from farm A. One strain isolated from iceberg lettuce was serotype O:2, but the other strains did not agglutinate with O:1–O:6 antisera. The PFGE patterns of environmental strains did not match the PFGE pattern of the outbreak strain.

DISCUSSION

In this widespread outbreak, *Y. pseudotuberculosis* O:3 infections were strongly associated with consumption of iceberg lettuce, which 71% of case patients reported having eaten. The frequency of having eaten iceberg lettuce suggested a doseresponse relationship with illness, likely reflecting increased

probability of having eaten contaminated lettuce. Although no iceberg lettuce implicated by the trace-back investigation was available for culture, subsequent environmental investigation identified a plausible contamination mechanism—that is, through irrigation water or direct contamination from animal feces at the source.

Y. pseudotuberculosis is considered to be a foodborne pathogen, but it has rarely been isolated from foods [16, 17], and foodborne transmission has not been previously documented. In the few reported community outbreaks, fresh produce [1], vegetable juice [5], untreated surface water [18], and homogenized milk [7] were suspected as possible sources of infection, on the basis of descriptive data, but no definitive link with a specific vehicle was established. The present study is the first report of a *Y. pseudo-tuberculosis* outbreak in which a specific food has been implicated in a population-based, controlled epidemiologic study and traced back to be the source of contamination.

The outbreak was detected rapidly because of ongoing laboratory-based surveillance and serotype analysis; molecular subtype analysis linked geographically dispersed and apparently unrelated cases. Population-based registries facilitated both finding of case subjects and enrolling of a representative control group for the case-control study. Although the role of bias and confounding should be considered [19], the findings are un-

No. of times eaten during 2 weeks ^a	Case patients $(n = 38)$	Control subjects $(n = 69)^{b}$	MOR (95% CI)	P for trend
>5	11 (29)	2 (3)	11.8 (1.5–532.4)	<.001
3–5	10 (26)	11 (16)	9.5 (1.2–433.7)	
1–2	6 (16)	17 (25)	1.1 (0.2–5.3)	
0	11 (29)	39 (56)	Referent	

Table 3. Dose-response relationship between frequency of iceberg lettuce consumption and odds of *Yersinia pseudotuberculosis* infection.

NOTE. Data are no. of persons with characteristic (%), unless otherwise noted. CI, confidence interval; MOR, matched odds ratio.

^a The 2 weeks before onset of illness, for case patients, and the 2 weeks before the interview, for control subjects.

^b Data regarding iceberg lettuce consumption were not available for 7 control subjects.

likely to reflect differential recall among case patients and control subjects. To reduce the potential for recall bias among control subjects, we obtained food histories that represent food preferences. Since eating habits are unlikely to vary substantially over a relatively short time, food preferences were considered to be less susceptible to information bias. It also seems improbable that differential recall or an unknown confounding factor would be consistently associated with increasing frequency of lettuce consumption.

Limited data are available to evaluate possible seasonality of lettuce consumption. According to the records of the Finnish Central Union of Agricultural Producers and Forest Owners and the National Board of Customs, domestically produced iceberg lettuce is generally available June–October and sometimes during November. The relative proportion of domestic and imported iceberg lettuce consumed varies by month. During the winter months, the iceberg lettuce is imported, but, overall, the total monthly consumption of iceberg lettuce in Finland is relatively constant throughout the year. Although the interviewers could not be blinded to case-control status, the questionnaire was structured to minimize bias. The participants had no reason to suspect the association, because the vehicle was previously unknown and the hypothesis was not disclosed in the media during the investigation.

Few countries conduct national surveillance for *Y. pseudo-tuberculosis* infections, and the epidemiology of these infections is poorly understood [10]. In Finland, 30–40 culture-confirmed *Y. pseudotuberculosis* infections are identified annually (incidence, 0.6–0.8/100,000 persons/year). However, culture-confirmed infections probably represent only a proportion of the disease burden, because clinical diagnosis is difficult, and routine stool cultures may not detect the organism [20]. Some patients have only fever with abdominal pain or mild symptoms [2], and stool cultures may not be requested. In this outbreak, the actual number of ill persons was probably much higher, as suggested by the identified clusters of patients who underwent appendectomies. Our findings emphasize the importance of

testing stool specimens for *Yersinia* species in patients with febrile gastroenteritis and abdominal pain.

The exact mechanism for contamination of the iceberg lettuce remains unknown, but it is likely to have resulted from use of irrigation water contaminated with animal feces. It could also have occurred from direct contamination by animal feces or from surface water runoff. The trace-back investigation suggested that contamination was probably intermittent and not uniform. Wildlife had access to irrigation water sources and fields, and large quantities of roe deer feces were found in both areas. In Europe, Japan, and North America, Y. pseudotuberculosis is frequently isolated from many domestic and wild animals [17, 21, 22]. Subclinical infections and asymptomatic carriage are common among deer, making it a possible animal reservoir. Outbreaks of diarrheal illness also have been reported, with serotype O:3 as the most commonly isolated strain [23]. Although the recovered environmental isolates were not related to the outbreak, the presence of Y. pseudotuberculosis bacteria in iceberg lettuce and irrigation water samples indicated fecal contamination from infected animals, supporting the hypothesis that contamination occurred at the farm before distribution. The bacteria can survive in surface water [4, 24], and it is conceivable that other types of fresh produce irrigated with water from the same sources may also become contaminated.

Fresh produce may become contaminated during irrigation, harvesting, packing, shipping, and processing [25]. Foodborne outbreaks are increasingly being associated with fresh produce contaminated before distribution. Lettuce has been implicated as the vehicle of infection for many infectious agents, such as *Escherichia coli* O157:H7 [26, 27], *Shigella sonnei* [28–30], and hepatitis A virus [31]. Large surface area, features that favor bacterial attachment, and difficulty of cleaning properly may make lettuce an efficient vehicle of infection. Internal contamination of the lettuce is also a possibility [32]. Transportation and storage of lettuce at low temperatures may be particularly favorable for survival and growth of *Yersinia* species [33].

This outbreak underscores the need to implement measures

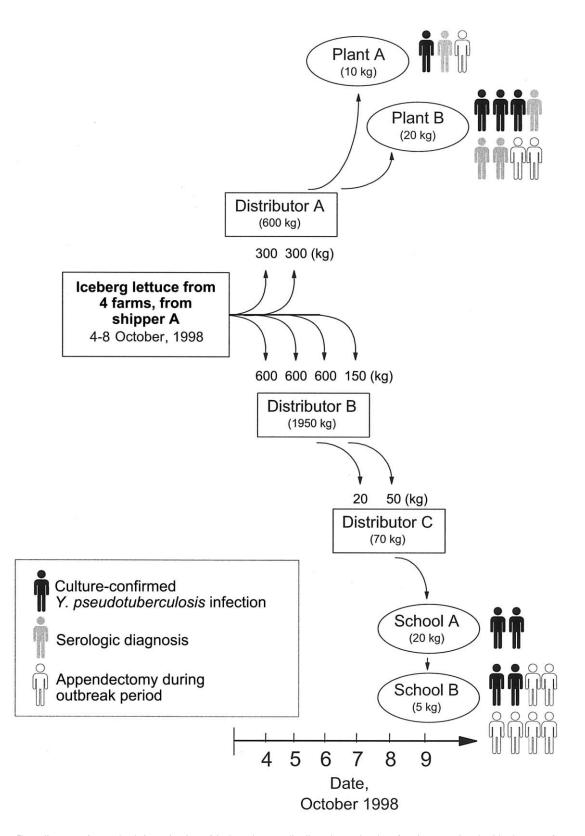


Figure 3. Flow diagram of trace-back investigation of iceberg lettuce distributed to 4 lunch cafeterias associated with clusters of case patients and patients who underwent appendectomies. "Serologic diagnosis" indicates an elevated level of specific IgM antibodies against *Yersinia pseudo-tuberculosis* 0:3, by EIA. At plant B, the 2 patients who underwent appendectomies during the outbreak period also had serologic evidence of recent *Y. pseudotuberculosis* 0:3 infection.

at the farm level to prevent Y. pseudotuberculosis contamination, by use of clean or treated irrigation water and by protecting the fields and water sources from animals accessing them, by the addition of fences. Naturally, raw produce should always be washed before consumption, but, since lettuce is not usually cooked before being eaten, it may be difficult for consumers to protect themselves. The implications for regulations concerning lettuce production and shipping conditions, as well as international trade, are also important. In the European Union, products of animal origin that have potential for transmission of zoonoses are strictly regulated, and hazardous batches can therefore be rapidly traced and recalled from the market [34]. Since these regulations do not apply to foodstuffs of nonanimal origin, production of and shipping conditions for fresh produce are less controlled, and records of origin may be inaccurate. The trace-back efforts of this investigation were hampered by incomplete documentation.

Contamination of commercially distributed fresh produce is increasingly causing widespread foodborne outbreaks, which are difficult to detect and trace back to the source [27, 28, 31, 35]. Our investigation has provided evidence that *Y. pseudotuberculosis* is also an emerging pathogen with the capacity to cause widespread foodborne outbreaks associated with substantial morbidity, including unnecessary appendectomies and postinfectious complications. Most reported cases of *Y. pseudotuberculosis* infection in Finland are not part of recognized outbreaks. However, some of these, which appear to be sporadic, may be part of unrecognized outbreaks caused by intermittent or low-level contamination of fresh produce.

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References

- Tertti R, Granfors K, Lehtonen OP, et al. An outbreak of Yersinia pseudotuberculosis infection. J Infect Dis 1984; 149:245–50.
- Tertti R, Vuento R, Mikkola P, Granfors K, Mäkelä AL, Toivanen A. Clinical manifestations of *Yersinia pseudotuberculosis* infection in children. Eur J Clin Microbiol Infect Dis **1989**;8:587–91.
- Fukushima H, Gomyoda M, Ishikura S, et al. Cat-contaminated environmental substances lead to *Yersinia pseudotuberculosis* infection in children. J Clin Microbiol 1989; 27:2706–9.
- Fukushima H, Gomyoda M, Shiozawa K, Kaneko S, Tsubokura M. Yersinia pseudotuberculosis infection contracted through water contaminated by a wild animal. J Clin Microbiol 1988; 26:584–5.
- Inoue M, Nakashima H, Ueba O, et al. Community outbreak of Yersinia pseudotuberculosis. Microbiol Immunol 1984; 28:883–91.
- Nakano T, Kawaguchi H, Nakao K, Maruyama T, Kamiya H, Sakurai M. Two outbreaks of *Yersinia pseudotuberculosis* 5a infection in Japan. Scand J Infect Dis **1989**; 21:175–9.

- Nowgesic E, Fyfe M, Hockin J, et al. Outbreak of *Yersinia pseudotu-berculosis* in British Columbia—November 1998. Can Commun Dis Rep 1999; 25:97–100.
- Black RE, Jackson RJ, Tsai T, et al. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. N Engl J Med **1978**; 298:76–9.
- 9. Tauxe RV, Vandepitte J, Wauters G, et al. *Yersinia enterocolitica* infections and pork: the missing link. Lancet **1987**; 1:1129–32.
- Ostroff SM, Kapperud G, Hutwagner LC, et al. Sources of sporadic <u>Yersinia enterocolitica</u> infections in Norway: a prospective case-control study. Epidemiol Infect 1994; 112:133–41.
- Farmer JJ 3rd. Enterobacteriaceae: introduction and identification. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology. 6th ed. Washington, DC: ASM Press, 1995: 438–50.
- Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; 33:2233–9.
- Lukinmaa S, Schildt R, Rinttilä T, Siitonen A. Salmonella Enteritidis phage types 1 and 4: pheno- and genotypic epidemiology of recent outbreaks in Finland. J Clin Microbiol 1999; 37:2176–82.
- Niskanen T, Fredriksson-Ahomaa M, Korkeala H. Yersinia pseudotuberculosis with limited genetic diversity is a common finding in tonsils of fattening pigs. J Food Prot 2002; 65:540–5.
- Hannu T, Mattila L, Nuorti JP, et al. Reactive arthritis after an outbreak of *Yersinia pseudotuberculosis* serotype O:3 infection. Ann Rheum Dis 2003; 62:866–9.
- Schiemann DA. Yersinia enterocolitica and Yersinia pseudotuberculosis. In: Doyle M, ed. Foodborne bacterial pathogens. New York: Marcel Dekker, 1989:601–58.
- Chiesa C, Pacifico L, Nanni F, Renzi AM, Ravagnan G. Yersinia pseudotuberculosis in Italy: attempted recovery from 37,666 samples. Microbiol Immunol 1993; 37:391–4.
- Inoue M, Nakashima H, Ishida T, Tsubokura M. Three outbreaks of <u>Yersinia pseudotuberculosis</u> infection. Zentralbl Bakteriol Mikrobiol Hyg [B] 1988; 186:504–11.
- Schlesselman J. Case-control studies, design, conduct, analysis. New York: Oxford University Press, 1982:124–43.
- Leino R, Granfors K, Havia T, Heinonen R, Lampinen M, Toivanen A. Yersiniosis as a gastrointestinal disease. Scand J Infect Dis 1987; 19: 63–8.
- Fukushima H, Gomyoda M. Intestinal carriage of *Yersinia pseudotu-berculosis* by wild birds and mammals in Japan. Appl Environ Microbiol 1991; 57:1152–5.
- Toma S. Human and nonhuman infections caused by *Yersinia pseudotuberculosis* in Canada from 1962 to 1985. J Clin Microbiol 1986; 24:465–6.
- 23. Sanford SE. Outbreaks of yersiniosis caused by *Yersinia pseudotuber-culosis* in farmed cervids. J Vet Diagn Invest **1995**; 7:78–81.
- Sato K, Komazawa M. Yersinia pseudotuberculosis infection in children due to untreated drinking water. Contrib Microbiol Immunol 1991; 12:5–10.
- Beuchat LR, Ryu JH. Produce handling and processing practices. Emerg Infect Dis 1997; 3:459–65.
- Ackers ML, Mahon BE, Leahy E, et al. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. J Infect Dis **1998**; 177:1588–93.
- Hilborn ED, Mermin JH, Mshar PA, et al. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Arch Intern Med **1999**; 159:1758–64.
- Davis H, Taylor JP, Perdue JN, et al. A shigellosis outbreak traced to commercially distributed shredded lettuce. Am J Epidemiol 1988; 128: 1312–21.

- 29. Kapperud G, Rorvik LM, Hasseltvedt V, et al. Outbreak of *Shigella sonnei* infection traced to imported iceberg lettuce. J Clin Microbiol **1995**; 33:609–14.
- Frost JA, McEvoy MB, Bentley CA, Andersson Y. An outbreak of *Shigella sonnei* infection associated with consumption of iceberg lettuce. Emerg Infect Dis **1995**; 1:26–9.
- Rosenblum LS, Mirkin IR, Allen DT, Safford S, Hadler SC. A multifocal outbreak of hepatitis A traced to commercially distributed lettuce. Am J Public Health 1990; 80:1075–9.
- 32. Solomon EB, Yaron S, Matthews KR. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce

plant tissue and its subsequent internalization. Appl Environ Microbiol **2002**; 68:397–400.

- Schiemann DA, Wauters G. *Yersinia*. In: Vandederzant C, Splittstoesser DF, eds. Compendium of methods for the microbiological examination of foods. Washington, DC: American Public Health Association, 1992: 433–50.
- Klapwijk PM, Jouve JL, Stringer MF. Microbiological risk assessment in Europe: the next decade. Int J Food Microbiol 2000; 58:223–30.
- 35. Tauxe R, Kruse H, Hedberg C, Potter M, Madden J, Wachsmuth K. Microbial hazards and emerging issues associated with produce—a preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. J Food Prot 1997; 60:1400–8.