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Outbreak of *Shigella sonnei* Infection Traced to Imported Iceberg Lettuce

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In the period from May through June 1994, an increase in the number of domestic cases of *Shigella sonnei* infection was detected in several European countries, including Norway, Sweden, and the United Kingdom. In all three countries epidemiological evidence incriminated imported iceberg lettuce of Spanish origin as the vehicle of transmission. The outbreaks shared a number of common features: a predominance of adults among the case patients, the presence of double infections with other enteropathogens, and the finding of two dominant phage types among the bacterial isolates. In Norway 110 culture-confirmed cases of infection were recorded; more than two-thirds (73%) were adults aged 30 to 60 years. A nationwide case-control study comprising 47 case patients and 155 matched control individuals showed that the consumption of imported iceberg lettuce was independently associated with an increased risk of shigellosis. Epidemiological investigation of a local outbreak incriminated iceberg lettuce from Spain, consumed from a salad bar, as the source. The presence of shigellae in the suspected food source could not be documented retrospectively. However, high numbers of fecal coliforms were detected in iceberg lettuce from patients' homes. Three lettuce specimens yielded salmonellae. The imported iceberg lettuce harbored *Escherichia coli* strains showing resistance to several antimicrobial agents, including ampicillin, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole. During the outbreak it is likely that thousands of Norwegians and an unknown number of consumers in other countries were exposed to coliforms containing antibiotic resistance genes.

Illness caused by food contaminated with infectious or toxigenic microorganisms is a major cause of suffering and a very significant cause of death throughout the world (2, 27). Infections caused by *Shigella* species continue to be an important cause of diarrheal disease (26). In early parts of the 20th century, *Shigella dysenteriae* strains were the primary cause of shigellosis. *S. dysenteriae* has almost completely disappeared from developed countries, replaced first by *Shigella flexneri* and more recently by *Shigella sonnei*. The bacterium is frequently spread by person-to-person transmission by the fecal-oral route, but it may also be spread indirectly by fecal contamination of food or water (26).

Many foodborne and waterborne outbreaks of shigellosis are reported each year. Previously described outbreaks indicate that lettuce, either intact or shredded, can serve as an effective vehicle for *S. sonnei* (5, 18). During June 1994 a nationwide outbreak of *S. sonnei* infection was detected in Norway (10). Epidemiological evidence suggested that imported iceberg lettuce was the vehicle of transmission. At about the same time, outbreaks with similar characteristics were independently reported from other European countries, including Sweden and the United Kingdom, both of which incriminated imported iceberg lettuce on the basis of epidemiological investigations (4, 22). The purpose of this article is to describe the outbreak in Norway and the investigations which led to identification of the source of infection.

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Description of the outbreak. In the first 2 weeks of June 1994 the National Notification System for Infectious Diseases received an increasing number of reports of *S. sonnei* infection. A majority of the cases were in adults who had not traveled abroad in the weeks prior to the onset of symptoms. Since reports were obtained from several geographically widespread counties, it was presumed that a common-source outbreak with a nationwide distribution was taking place. At the same time the Norwegian Food Control Authority received reports from 15 municipal food inspection services throughout the country describing outbreaks of gastroenteritis, many of which were characterized by an unusually high attack rate. In several of these outbreaks, epidemiological evidence suggested that consumption of imported iceberg lettuce was the source of infection. Although *S. sonnei* was recovered from patients in some of the outbreaks, attempts to isolate shigellae from the suspected food source proved unsuccessful. However, high numbers of fecal coliforms (up to 80,000 CFU/g) were detected in imported iceberg lettuce from patients' homes, suggesting heavy fecal contamination. By 16 June 1994 the number of reported cases had reached 76, and the following day a public health warning was released in which the consumers were advised not to eat imported iceberg lettuce. Norwegian iceberg lettuce was not generally available on the market when the outbreak began and was not associated with illness. By the middle of June, however, the market was dominated by Norwegian products because of ordinary restrictions on the quantities of imported lettuce, and at a meeting on 16 June the vegetable importers agreed to recall any remaining iceberg lettuce produced abroad. By week 27 of 1994 the outbreak had come to an end (Fig. 1).

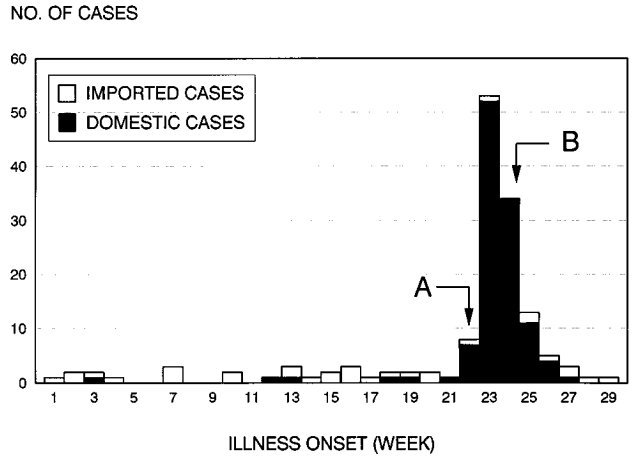

FIG. 1. Culture-confirmed cases of *S. sonnei* infection by week of illness onset and place of infection in Norway in 1994. Black bars indicate case patients with no reported history of traveling abroad prior to the onset of illness (domestic cases). Open bars indicate case patients who developed symptoms abroad or shortly after their return home (imported cases). A, the last date when iceberg lettuce was imported. B, the week when the public health warning was issued and the incriminated iceberg lettuce was withdrawn from the market.

MATERIALS AND METHODS

National notification system. Since 1975 Norway has maintained a national notification system for infectious diseases, including shigellosis (8). Data on culture-confirmed cases are reported consecutively by all medical microbiological laboratories in the country. For each reported case, epidemiological, bacteriological, and clinical information is recorded. The bacteriological information is adjusted according to the data received from the Reference Laboratory at the National Institute of Public Health, which verifies and types bacterial isolates. In addition to the laboratory-based notification system, the primary health service reports weekly the total number of consultations for gastrointestinal infections, irrespective of the causal agent.

Case-control study. We defined a case patient as a patient with culture-confirmed *S. sonnei* infection with no reported history of traveling abroad during the last week prior to the onset of his or her illness and who was reported to the national notification system during weeks 21 through 27 of 1994. If cultures of stool from more than one member of a household yielded a *Shigella* sp., only the first identified case patient was enrolled in the study. Each case patient was matched by age, sex, and geographic area with six control individuals selected from the Norwegian Population Registry (a governmental record of all residents which is updated on a quarterly basis). Matching was obtained by selecting individuals in the registry who were closest in age to the case patient and who lived in the same county. The case patients and their controls were rarely more than 2 weeks apart in age. Exclusion criteria for controls were diarrhea or abdominal pain with fever since 1 May 1994 and travel abroad during the first week of June 1994. Case patients and controls were mailed a structured questionnaire which covered the consumption of vegetables, the consumption of imported food items, diarrheal illness among household members, and travels abroad. Case patients were questioned about their exposures in the week prior to the onset of illness, whereas controls were questioned about their exposures in the first week of June 1994. In addition, case patients were inquired about the duration of their symptoms, the number of medical visits, antimicrobial treatment, hospitalization, and the time lost from work or school.

Univariate analyses of dichotomous variables were performed by using the procedure for matched data sets (25) in the computer program Epi Info (Centers for Disease Control and Prevention, Atlanta, Ga.). Conditional logistic regression was implemented for univariate analysis of continuous variables and for multivariate analyses (25) by using the computer program Egret (Statistics and Epidemiology Research Corporation, Seattle, Wash.). The results are reported as matched odds ratios (ORs) with 95% confidence intervals and two-tailed *P* values.

Bacteriological examination of iceberg lettuce. A 10-g sample of each specimen was homogenized for 1 min in 90 ml of phosphate-buffered peptone water (pH 7.0) with a Colworth 400 stomacher, and one loopful was subsequently streaked onto bromothymol-blue saccharose (BS) agar, desoxycholate-citrate (DC) agar (Oxoid Ltd., Basingstoke, England), and lactose-saccharose-urea (LSU) agar (E. Merck, Darmstadt, Germany). The homogenates were incubated at 30°C for 18 to 20 h for resuscitation, and 10 ml was then transferred to 90 ml of selenite broth (Biokar Diagnostics, Beauvais, France) and the mixture was incubated at 37°C for 18 to 20 h for selective enrichment; this was followed by plating onto DC and BS agars. In addition, a 10-g sample of each specimen was homogenized in GN Broth Hajna (Difco Laboratories, Detroit, Mich.), and the mixture was incubated at 30°C for 5 to 6 h before plating onto DC and BS agars. Furthermore, 50-g samples were washed gently in a sterile plastic bag with 50 ml of peptone water, the mixture was centrifuged at 3,000 × *g* for 15 min, and one loopful of the pellet was streaked onto DC and BS agar plates. All plates were incubated at 37°C for 18 to 24 h. Suspect colonies were subcultured onto LSU and BS agar plates for further morphological inspection. A primary biochemical screening was carried out by the three-tube method described by Lassen (14). Shigellae were identified by established criteria (23). All suspect isolates were sent to the National Institute of Public Health for verification. Fecal coliforms were enumerated by a standard plate count procedure by the method recommended by the Nordic Committee on Food Analysis (21).

Plasmid profile analysis. Eleven clinical *S. sonnei* isolates, one from each of the counties where outbreak-related cases of infection were recorded, were subjected to plasmid profile analysis. Also examined were 2 human isolates from the outbreak in Sweden and 12 epidemiologically unrelated control strains isolated from Norwegian patients in 1992 and 1993. The isolates were cultured overnight at 37°C in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 0.6% yeast extract. The plasmid profile was investigated by a small-scale modification of the alkaline lysis technique of Birnboim and Doly (3) as described by Maniatis et al. (17); this was followed by electrophoresis for 3 h at 120 V on a 0.7% horizontal agarose gel in Tris-borate buffer. Plasmid DNAs from *Escherichia coli* VA 517 (16) and J5 (RI) were included as molecular weight standards on each gel.

Phage typing. Phage typing was done at the Swedish Institute for Infectious Disease Control, Stockholm, by the typing scheme described by Kallings et al. (13).

Antimicrobial susceptibility testing. Fifty-nine single-colony isolates of fecal coliform bacteria from 13 heads of imported iceberg lettuce (one to nine isolates per specimen) were studied. Also examined were 11 clinical *S. sonnei* isolates, 1 from each of the counties where the outbreak was described. Coliforms were kept at −70°C for 2 months and were checked for purity before species determination, which was accomplished with the API 32 Rapid and API 20 E systems (Bio Merieux, Marcey-l'Etoile, France). The coliforms were screened for antibiotic susceptibility on PDM Antibiotic Sensitivity Medium (AB Biodisk, Solna, Sweden) by a standard agar diffusion method with commercial antibiotic tablets (Neo-Sensitabs; A/S Rosco, Taastrup, Denmark). Plates were incubated at 37°C for 18 to 20 h, and the isolates were classified by established criteria (19, 20). Except for a few isolates which were resistant to ampicillin only, all isolates showing evidence of resistance to any of the antimicrobial agents tested were reexamined by the E test (AB Biodisk) to confirm the results (9). The antibiograms of the 11 *S. sonnei* isolates were determined by the E test without prior screening. All isolates were tested for their susceptibilities to the following chemotherapeutic agents: ampicillin, amoxicillin-clavulanate, mecillinam, cefuroxime, cefoxitin, cefotaxime, ceftazidime, imipenem, gentamicin, tobramycin, ciprofloxacin, and trimethoprim-sulfamethoxazole. *Shigella* isolates were also tested against chloramphenicol.

RESULTS

Descriptive epidemiology. During the outbreak, a total of 110 culture-confirmed cases of *S. sonnei* infection among individuals with no reported history of traveling abroad were recorded (Fig. 1). The number of cases of infection reached a peak in week 23 of 1994 and declined when imported iceberg lettuce was no longer available on the market, indicating that the discrete source of infection had been removed. Fifty-two of the patients were males and 58 were females (male-to-female ratio, 0.90). The ages of the patients ranged from 9 to 82 years (median, 40 years; mean, 38 years). However, more than two-thirds (73%) of all case patients were adults aged 30 to 60 years. Only 10 case patients were less than 20 years of age (Fig. 2). Case patients were reported from 11 of Norway's 19 counties.

From two patients, salmonellae were recovered from the same stool specimen which yielded *S. sonnei*. The *Salmonella* serovars encountered were *Salmonella heidelberg* and *Salmonella thompson*. No significant increase in the number of reported cases owing to *Salmonella* or *Campylobacter* spp. was recorded during the outbreak. However, the primary health service reported an increase in the total number of consultations for gastrointestinal infections (Fig. 3). This cannot be explained by increased awareness, since it occurred before the public health warning was issued.

Case-control study. During week 25 of 1994 the National

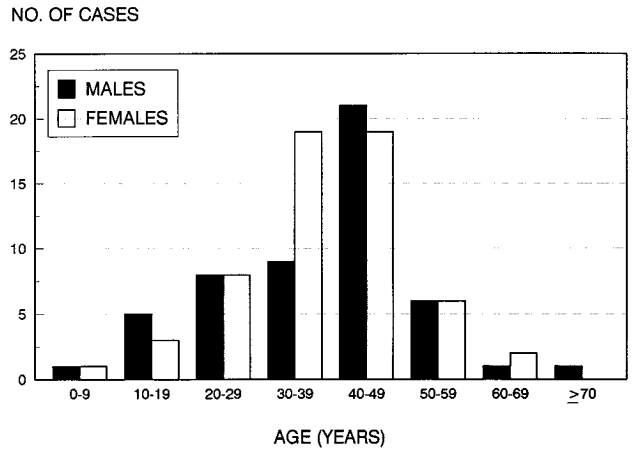

FIG. 2. Age and sex distributions of 110 patients with culture-confirmed *S. sonnei* infections reported during the outbreak in Norway in 1994.

Institute of Public Health conducted a case-control study designed to identify the risk factors for the outbreak-related *S. sonnei* infections. A structured questionnaire was mailed to the first 50 patients reported to have culture-confirmed infections. Forty-eight patients (98%) answered the questionnaire; one patient was excluded because she had been abroad prior to the onset of illness. Thus, 43% of all eligible case patients were enrolled in the study. Study enrollees were similar to the case patients recorded by the notification system with respect to age, sex, and geographical distribution. A mean of 22 days elapsed between the onset of illness and completion of the questionnaire (median, 21 days; range, 12 to 55 days).

Of 288 control individuals contacted, 182 (63%) answered the questionnaire. Twenty were excluded because of recent diarrheal illness, and seven were ineligible for enrollment because they had been abroad during the period covered by the questionnaire. The remaining 155 individuals satisfied the criteria for a control. Consequently, data for 47 case patients and 155 matched controls were included in the final data set.

By univariate analysis, consumption of iceberg lettuce in the week prior to the onset of illness was strongly associated with an increased risk of *S. sonnei* infection (OR = 63.8; $P < 0.0001$). Forty-six (98%) of 47 case patients but only 35 (24%) of 148 controls reported this exposure (Table 1). Case patients were significantly more likely than their controls to have eaten iceberg lettuce produced in a foreign country (OR = 33.3; $P < 0.0001$). However, only 48 (59%) of the respondents who reported iceberg lettuce consumption were able to recall whether it was imported or domestically produced. Conditional linear logistic regression analysis confirmed that consumption of imported iceberg lettuce was an independent risk factor. More cases than controls had eaten radish (OR = 2.7; $P = 0.05$), but this exposure was not independently associated with illness in the multivariate analysis. Consumption of Chinese lettuce, which is commonly eaten instead of iceberg lettuce, was more frequently reported by controls than by case patients (OR = 0.4; $P = 0.02$). No significant associations were detected for 13 other raw vegetables. Consumption of imported poultry, red meat products, or strawberries was not associated with disease (Table 1).

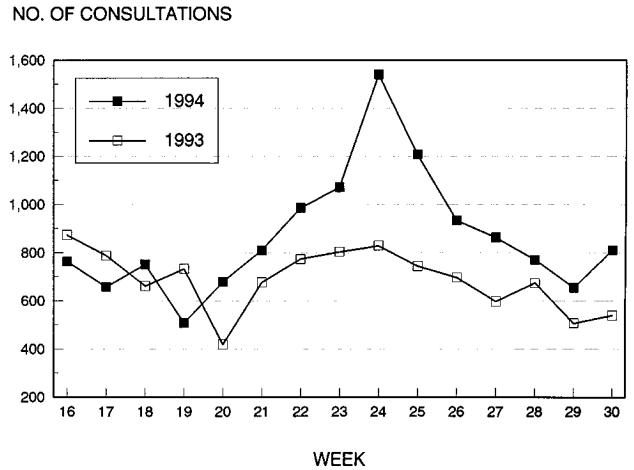

FIG. 3. Total number of consultations for acute gastroenteritis by week reported by the primary health service in Norway in 1993 and 1994.

Twenty case patients (43%) reported diarrheal disease among household members in the days after they became ill, whereas only two controls (1%) described such illness (OR = 17.5; $P < 0.001$). In nine households with case patients (18%), *S. sonnei* was reportedly isolated from other household members as well.

Epidemiological investigations of local outbreaks. During June 1994 the food control authorities in Midt-Rogaland County recorded 45 outbreaks of gastroenteritis. Investigations revealed that consumption of imported iceberg lettuce was a common feature. Of 304 people who had eaten iceberg lettuce, 276 developed disease. Iceberg lettuce of Spanish origin was incriminated in several of the outbreaks, one of which is described below. However, because of incomplete labelling, the possibility that some outbreaks were caused by French products could not be excluded.

During the period 31 May through 13 June 1994, 85 of 322 employees at a company in southwestern Norway developed symptoms of gastroenteritis. Stool specimens from 12 patients were cultured, and one specimen yielded *S. sonnei*. All patients had eaten food from a salad bar in the company's canteen, where a variety of raw vegetables including iceberg lettuce was served. A total of 65 patients and 40 healthy control persons, all of whom had visited the salad bar on 9 or 10 June 1994, were interviewed about the consumption of raw vegetables on the 2 days concerned. Consumption of iceberg lettuce was strongly associated with an increased risk of disease, whereas consumption of the other six available raw vegetables was not. In all, 62 (95%) of the 65 case patients but only 1 (3%) of the 40 controls reported having eaten iceberg lettuce (OR = 806.0, $P < 0.0001$). The iceberg lettuce was traced to a wholesale dealer who reported that the lot in question had been imported from Spain.

Economic impact. Economic impact data were recorded for the 47 case patients enrolled in the case-control study. The patients were symptomatic for a mean of 15 days (median, 14 days; range, 7 to 28 days), and a mean of 5 days was lost from work or school (median, 4 days; range, 0 to 28 days). However, 9 believed that their symptoms had not resolved when the questionnaire was completed. The patients visited a physician on average two times (median, two times; range, zero to six times), and eight of the case patients were hospitalized for a mean of 4 days (median, 3 days; range, 1 to 8 days). Eleven patients received antimicrobial treatment for their infections (one patient was unsure).

Bacteriological examination of iceberg lettuce. On 16 June 1994 a letter of information was forwarded to all municipal food inspection services in Norway. The letter prompted them to submit samples of imported iceberg lettuce to the Norwe-

TABLE 1. Univariate analysis of selected dietary risk factors for shigellosis in Norway in 1994

Risk factor	No. (%) of exposed individuals/total no. of respondents[a]		OR[b]	95% Confidence interval	*P* value
	Case patients (n = 47)	Controls (n = 155)			
Raw vegetables					
Bean sprouts	0/46 (0.0)	4/153 (2.6)	NC[c]	NC	NC
Cabbage	4/46 (8.7)	30/149 (20.1)	0.36	0.1–1.1	0.11
Carrots	18/46 (39.1)	81/149 (54.4)	0.55	0.3–1.1	0.14
Cauliflower	4/44 (9.1)	21/151 (13.9)	0.63	0.2–1.9	0.53
Celery	7/43 (16.3)	26/151 (17.2)	0.94	0.4–2.3	0.93
Chinese lettuce	14/46 (30.4)	78/144 (54.2)	0.36	0.2–0.8	0.017
Cucumber	41/47 (87.2)	127/153 (83.0)	1.60	0.5–4.7	0.55
Iceberg lettuce	46/47 (97.9)	35/148 (23.6)	63.83	10.0–406.7	<0.0001
Leek	6/44 (13.6)	19/149 (12.8)	1.24	0.5–3.2	0.82
Lettuce	21/46 (45.7)	73/148 (49.3)	0.85	0.5–1.6	0.73
Mushroom	3/44 (6.8)	13/152 (8.6)	0.89	0.2–3.9	0.84
Onion	11/43 (25/6)	48/152 (31.6)	0.82	0.4–1.9	0.78
Radish	10/44 (22.7)	18/153 (11.8)	2.66	1.0–6.9	0.046
Red pepper	23/44 (52.3)	91/152 (59.9)	0.64	0.3–1.4	0.33
Rutabaga	0/45 (0.0)	26/152 (17.1)	NC	NC	NC
Tomato	39/47 (83.0)	129/150 (86.0)	0.86	0.4–2.0	0.89
Other vegetables	2/42 (4.8)	3/147 (2.0)	1.82	0.3–11.8	0.92
Imported foods					
Iceberg lettuce	34/35 (97.1)	6/127 (4.7)	33.25	5.8–189.5	<0.0001
Poultry	1/43 (2.3)	8/153 (5.2)	0.40	0.1–3.0	0.59
Red meat	6/43 (14.0)	20/151 (13.2)	0.83	0.3–2.3	0.92
Strawberries	5/42 (11.9)	33/153 (21.6)	0.49	0.2–1.4	0.23

[a] Denominators exclude individuals for whom risk factor values were missing.
[b] Matched odds ratio.
[c] NC, not calculable.

gian College of Veterinary Medicine. During the last 2 weeks of June 1994 a total of 57 specimens were received. Thirty-nine samples were obtained from patients with culture-confirmed shigellosis or with symptoms compatible with the diagnosis who had eaten iceberg lettuce in the days before illness. An additional 18 samples were obtained from retail outlets. Most specimens were forwarded without cooling. *Shigella* spp. were not recovered from any of the samples. Likewise, a large number of samples examined by local food inspection laboratories were negative for shigellae. However, three specimens yielded salmonellae. One of the isolates belonged to *Salmonella montevideo*; the serovar affiliations of the remaining two isolates were not determined. *S. montevideo* was not isolated from Norwegian patients during the outbreak.

Fecal coliforms were detected in 17 of 42 samples examined. Four samples contained 10^4 to 10^3 CFU/g, eight contained 10^3 to 10^2 CFU/g, while five were contaminated with 10^2 to 10^1 CFU/g. All but one of the samples which harbored coliforms were obtained from patients' kitchens.

Phage typing and plasmid profile analysis. Nine of the 13 outbreak-related isolates examined (1 from Sweden and 8 from Norway) showed two closely related plasmid profiles which differed only by possession of one large plasmid of 120 to 140 MDa (Fig. 4, lanes A to I). This cluster could be further subdivided into four phage types (PTs), of which PT 2 predominated, with five isolates being of PT 2. The remaining four outbreak-related isolates (one from Sweden and three from Norway), all belonging to PT 65, exhibited three distinct plasmid profiles, of which two differed only by the presence of the large plasmid (Fig. 4, lanes J to M). All 12 control strains showed plasmid profiles different from those of the outbreak-related isolates (only six are shown in Fig. 4).

Antimicrobial susceptibility. The 11 *S. sonnei* isolates were fully susceptible to all 13 antimicrobial agents tested, except that 1 isolate showed ampicillin resistance that was reversed by clavulanate. Of the 59 isolates of fecal coliforms examined, 52 were *E. coli*, 4 were *Klebsiella pneumoniae*, and 3 were *Enterobacter cloacae*. While no remarkable resistance was detected among the *Klebsiella* and *Enterobacter* isolates, 16 of the *E. coli* isolates were resistant to one or more agents. The following resistance patterns were detected: ampicillin only, three isolates; ampicillin plus trimethoprim-sulfamethoxazole, five isolates; ampicillin plus ciprofloxacin, one isolate; gentamicin plus

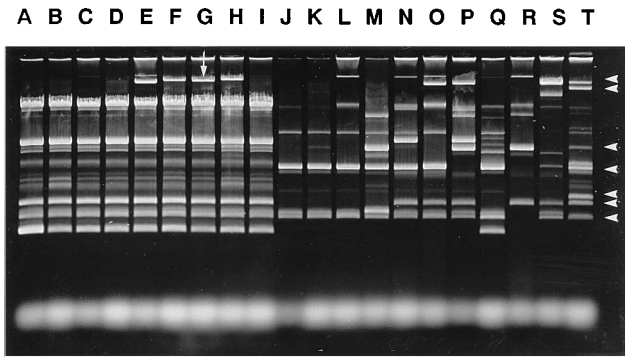

FIG. 4. Plasmid profiles of 19 *S. sonnei* isolates. Lanes A to M, 13 outbreak-related isolates; lanes N to S, 6 epidemiologically unrelated control isolates. The following PTs are represented: PT 2 (lanes A to E), PT 23 (lanes F and G), PT 3X (lane H), PT 75 (lane I), and PT 65 (lanes J to M). Control strains were not phage typed. The isolates in lanes A and J were obtained from Sweden; the remaining isolates were from Norway. The size markers in lane T are standard plasmids (from top to bottom, 62.0, 35.8, 4.8, 3.4, 2.0, 1.8, and 1.4 MDa). The vertical arrow in lane G indicates the position of the 120- to 140-MDa plasmid.

ciprofloxacin plus trimethoprim-sulfamethoxazole, one isolate; and trimethoprim-sulfamethoxazole only, six isolates. For both strains resistant to ciprofloxacin MICs were ≥32 mg/liter.

DISCUSSION

The outbreak in Norway probably constitutes only a small part of an international epidemic of unknown extent. In the period from May through June 1994, an increase in the number of domestic cases of *S. sonnei* infection was detected in several European countries, including Norway, Sweden, and the United Kingdom. In all three countries, epidemiological evidence incriminated imported iceberg lettuce as the vehicle of transmission. The results of our case-control study were supported by a parallel study conducted in England and Wales (22). Exploration of a local outbreak in Norway indicated that iceberg lettuce of Spanish origin was the source. Independent investigations in the United Kingdom and Sweden pointed in the same direction (1, 6). In accordance with our observation, a predominance of adults among the case patients was recorded (22), a circumstance which may be explained by a higher rate of lettuce consumption among adults relative to that among children. Another common feature was the detection of double infections with other enteric pathogens (1, 6). Eight of the 53 patients recorded in Sweden were infected with enterotoxigenic *E. coli* ($n = 4$), *Giardia lamblia* ($n = 2$), *Salmonella* sp. ($n = 1$), or *Campylobacter* sp. ($n = 1$) in addition to *S. sonnei* (1). In Norway and Sweden, a majority of the outbreak-related isolates belonged to PT 2 and PT 65, whereas PT 2 as well as PT L (closely related to PT 65) prevailed in the United Kingdom (22).

The presence of shigellae in the suspected food source could not be documented retrospectively, despite comprehensive bacteriological examinations. To our knowledge, no isolates of *Shigella* were obtained from iceberg lettuce in any of the countries involved in the outbreak. This was not unexpected, however, since the isolation of *Shigella* spp. from food as well as from feces is generally considered to be difficult (26). The bacterium competes poorly with other enteric flora and is easily overgrown. Current laboratory methods are relatively insensitive (26). Nevertheless, when inoculated into foods in high numbers, *Shigella* organisms may survive for periods ranging from less than 3 weeks to more than 3 months (26). *S. sonnei* has been shown to multiply in shredded lettuce and lettuce extract under certain conditions (5). The presence of even a small number of shigellae may be epidemiologically significant because of the low infective dose of these organisms. Unlike most other enteropathogenic bacteria, *Shigella* strains are known to cause infection in adults when they are taken at a dose of 10^1 to 10^2 cells (26). It is therefore likely that shigellae may have survived on the iceberg lettuce in numbers sufficient to cause disease but insufficient to allow detection by the laboratory methods used. Our attempts to recover the bacterium were further complicated by the fact that the samples had been stored for several days in patients' kitchens and were transported to the laboratory, often at ambient temperature. Moreover, the outer leaves of the lettuce, which are more exposed to contamination than the inner parts, were usually consumed prior to laboratory examination.

Humans and higher primates are the only known hosts for *Shigella* species (26), and the presence of shigellae in food or the environment is usually associated with fecal contamination from humans (26). It is tempting to suggest that the iceberg lettuce may have become contaminated in one of the following ways: (i) irrigation with incompletely treated sewage effluent or polluted drinking water, (ii) fertilization with sewage sludge or with manure containing human feces, or (iii) accidental flooding of cropland with polluted water following a period of heavy precipitation. Incomplete labelling of the iceberg lettuce has hampered identification of the responsible producer and has delayed full revelation of the events which led to the putative contamination. However, several observations support the suggestion that heavy fecal contamination occurred: (i) concomitant isolation of other enteropathogens in addition to *S. sonnei* from several patients, (ii) the finding of several phage types and plasmid profiles among the outbreak isolates, and (iii) the detection of salmonellae and high numbers of fecal coliforms in lettuce obtained from patients' homes.

Person-to-person transmission is common with *Shigella* infection (26). After initial infection from a contaminated food source, the disease readily spreads from person to person by direct or indirect fecal-oral transmission, which is enhanced by the low infective dose required. In our case-control study, diarrheal illness among household members was more frequently reported by case patients than by controls, and in nine households of case patients the organism was reportedly isolated from other family members. Although this may well reflect transmission within the family, it may also represent common source coinfection as well as selective recall of unrelated illness. Thus, it is difficult to draw conclusions about the extent of person-to-person transmission in the present outbreak. The possibility of intrafamily spread is not likely to have significantly affected our risk factor evaluation since only the first identified case patient from each household was enrolled in the case-control study.

The outbreak caused considerable morbidity in Norway. If the 47 study enrollees are representative of the 110 case patients reported to the national notification system, the reported infections led to an estimated 1,650 days of illness, 19 admissions to a hospital, 76 days of hospital stay, 550 days lost from work or school, 220 physician consultations, and 26 antimicrobial prescriptions among the 4.3 million Norwegians. These figures are absolute minimum values since several patients were still symptomatic when the questionnaire was completed. Moreover, the 110 culture-confirmed cases reported represent only a small fraction of the total number of infections that occurred. In *Salmonella* outbreaks, a ratio of reported to actual cases of 1:100 has been estimated (7).

Several phenotypic and genotypic methods have been used to differentiate *S. sonnei* isolates (26). Many of these typing methods are influenced by the loss or acquisition of plasmids. *S. sonnei* harbors a particular species of plasmid DNA of 120 to 140 MDa which is necessary for the expression of virulence (24, 29). This plasmid is known to be very unstable and is easily lost on subculture (15, 24), a fact which may explain the absence of this plasmid in several of our isolates (Fig. 4).

A major factor contributing to termination of the outbreak was effective legislative measures which prohibit the import of vegetables when Norwegian products are available on the market. Since regular restrictions on the quantities of imports were executed before the outbreak was recognized, at a time when the number of people affected by the outbreak was still increasing, it is likely that several hundred cases of infection were prevented. After the outbreak had been detected, this act was reinforced by a public health warning and voluntary withdrawal of all imported iceberg lettuce still for sale, but at that time the number of people affected by the outbreak was already on the decrease (Fig. 1).

Over the past couple of decades, foodborne diseases have increased considerably in the industrialized world and have reached epidemic proportions in several countries (2, 28). The reasons for the increase in reported foodborne disease are

probably the result of a combination of factors, including (i) more intensive farming and increased industrialization of all stages of food production, (ii) changes in food handling practices, eating habits, and the storage, distribution, and preparation of food, and (iii) more centralized food production and international trade in food (2, 28). There has been a systematic reduction in trade barriers with the establishment of a single market among the countries of the European Union and as a result of the North American Free Trade Agreement. These changes have brought with them new problems in food hygiene and have increased the risk of transboundary infections, with dissemination of pathogens to areas where the pathogens are not endemic as a possible consequence. The outbreak described here points to the need for a community-wide investigative body that could have taken the investigation to Spain and determined what went wrong in order to implement preventive measures. The outbreak also underlines the importance of laboratory-based surveillance in detecting outbreaks and emphasizes the need for intersectorial actions in outbreak investigations, with close cooperation between public health and food hygiene authorities.

A related problem of considerable public health concern is the global spread of antibiotic resistance genes (11). This problem is well documented and may have a significant effect on therapy, control measures, and, hence, the epidemiology of bacterial infections. Although all but one of the *S. sonnei* strains examined were fully susceptible to the antimicrobial agents tested, the imported iceberg lettuce harbored *E. coli* strains exhibiting resistance to several antimicrobial agents, the genetic determinants of which may be transferred to other bacterial species, including acknowledged pathogens (11). Some strains showed resistance patterns not previously described in Norway, where a restrictive policy regarding the introduction and use of antimicrobial agents has been pursued. Ciprofloxacin resistance has not been reported among clinical *E. coli* isolates from Norway so far (12), and gentamicin resistance is only exceptionally encountered. No indications of extended-spectrum β-lactamases were observed, but the methods used to detect such enzymes are suboptimal (9).

In conclusion, it is likely that during the outbreak shigellae as well as antibiotic resistance genes were disseminated to thousands of Norwegians and an unknown number of consumers in other countries. It is notable that while the *Shigella* outbreak attracted considerable attention, the spread of antimicrobial resistance would have remained unrecognized unless the outbreak had occurred.

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