Salmonella Typhimurium Infections Transmitted by Chlorine-pretreated Clover Sprout Seeds

John T. Brooks, Samantha Y. Rowe, Pamela Shillam, David M. Heltzel, Susan B. Hunter, Laurence Slutsker, Robert M. Hoekstra, Stephen P. Luby

American Journal of Epidemiology
Vol. 154, No. 11
Printed in U.S.A.

Salmonella Typhimurium Infections Transmitted by Chlorine-pretreated Clover Sprout Seeds

John T. Brooks,[1,2] Samantha Y. Rowe,[2] Pamela Shillam,[3] David M. Heltzel,[4] Susan B. Hunter,[2] Laurence Slutsker,[2] Robert M. Hoekstra,[5] and Stephen P. Luby[2]

Raw seed sprouts have caused numerous outbreaks of enteric infections. Presoaking seeds in a 20,000 mg/liter (ppm) calcium hypochlorite solution before sprouting is recommended to reduce bacterial contamination and infection risk. In 1999, the authors investigated an outbreak of *Salmonella* serotype Typhimurium infections in Colorado. In a case-control study, they matched 20 cases with 58 controls by age, sex, and telephone prefix; 10 (52%) of 19 cases and no controls recalled eating raw alfalfa-style sprouts in the 5 days before the patient's illness ($p < 0.00001$). Traceback implicated clover sprouts grown from seeds shared by two sprouters. The time period and region over which these sprouts were sold matched the occurrences of 112 culture-confirmed illnesses. Only one of the sprouters presoaked seeds as recommended, and fewer infections were attributable to this sprouter (0.29 vs. 1.13 culture-confirmed infections/50-pound (110.1-kg) bag of seed). After recall of the implicated sprouts and seed, *S.* Typhimurium illnesses declined. Contaminated raw clover sprouts can cause outbreaks of enteric illness. Presoaking contaminated seeds in a 20,000 mg/liter calcium hypochlorite solution reduces, but does not eliminate, the risk of infection. Until safer production methods are developed, persons eating raw sprouts continue to risk developing potentially serious gastrointestinal illness. *Am J Epidemiol* 2001;154:1020–8.

chlorine; disease outbreaks; gastroenteritis; *Salmonella typhimurium*; seeds; vegetables

Consumption of raw seed sprouts has caused outbreaks of *Salmonella*, *Bacillus cereus*, and *Escherichia coli* O157 infections in the United States and abroad (1). Although less than 10 percent of the US population routinely consumes sprouts (2, 3), they pose a special risk for young children, the elderly, and persons with weakened immune systems, for all of whom raw seed sprouts may be perceived as a healthy and beneficial food. Outbreaks have involved sprouts grown from alfalfa, radish, and cress seeds; mixtures of alfalfa, radish, soy, mustard, and clover seeds; and mung beans (1).

Transmission is usually ascribed to the presence on seeds of pathogenic bacteria, which multiply exponentially to infectious doses during the microbiologically favorable warm, moist, and nutrient-rich conditions of sprouting (4). In response to numerous sprout-associated outbreaks, in 1998, the Food and Drug Administration (FDA) issued an interim advisory on alfalfa sprouts, noting that concentrated chlorine solutions appeared to be potentially useful for decontaminating seeds before sprouting (5). No treatment has yet been shown to reliably prevent recovery of bacteria from sprouted seed (1). Soaking seeds in a 20,000 mg/liter (ppm) calcium hypochlorite solution for 15 minutes is the most effective chemical seed treatment currently approved (6–9).

In April 1999, public health officials in Colorado noted an increase in *Salmonella* serotype Typhimurium infections and subsequently notified the Centers for Disease Control and Prevention (CDC). For the first 16 weeks of 1999, the state reported 87 infections compared with 26, 51, and 45 infections during the same weeks in 1998, 1997, and 1996, respectively. Subtyping by pulsed-field gel electrophoresis (PFGE) demonstrated that 60 percent of these isolates shared a unique and indistinguishable PFGE pattern ("outbreak" PFGE pattern), suggesting that these infections were epidemiologically related.

Received for publication March 23, 2001, and accepted for publication August 8, 2001.

Abbreviations: CDC, Centers for Disease Control and Prevention; CDPHE, Colorado Department of Public Health and Environment; CI, confidence interval; FDA, Food and Drug Administration; OR, odds ratio; PFGE, pulsed-field gel electrophoresis.

[1] Epidemic Intelligence Service, Division of Applied Public Health Training, Epidemiology Program Office, Centers for Disease Control and Prevention, Atlanta, GA.
[2] Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA.
[3] Disease Control and Environmental Epidemiology Division, Colorado Department of Public Health and Environment, Denver, CO.
[4] Laboratory and Radiation Sciences Division, Colorado Department of Public Health and Environment, Denver, CO.
[5] Biostatistics and Information Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA.
Reprint requests to Dr. John T. Brooks, Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, MS A-38, Centers for Diseases Control and Prevention, 1600 Clifton Road NE, Atlanta, GA 30333 (e-mail: zud4@cdc.gov).

MATERIALS AND METHODS

Epidemiologic investigation

Salmonella infection is reportable in Colorado, and isolates from local laboratories and county health departments are routinely forwarded to the state laboratory for serotyping. Since January 1999, all *S.* Typhimurium isolates have been further subtyped by PFGE. To identify additional *S.* Typhimurium infections within Colorado, we notified county health departments of the outbreak and asked that they promptly report any new *Salmonella* infections and forward all isolates to the state laboratory. To identify infections outside Colorado, we contacted health officials in surrounding states and asked that they review recent surveillance data for *S.* Typhimurium and subtype by PFGE all *S.* Typhimurium isolates received since January 1999.

To determine the source of the outbreak, we conducted a case-control study. We defined a case as a diarrheal illness (three or more bowel movements during any 24-hour period) in a Colorado resident from whom *S.* Typhimurium with the outbreak PFGE pattern was isolated after April 15, 1999. We matched controls with cases by age group, sex, and telephone prefix and attempted to identify three controls for each case by systematic progressive telephone digit dialing. No cases under age 6 years were interviewed. We excluded potential controls who reported having diarrhea within the 30 days preceding the onset of illness in the case to whom they were matched or who had not resided in Colorado for the 5 days preceding the date of onset of illness in that case.

We developed a written questionnaire based on face-to-face, hypothesis-generating interviews with 13 Coloradans from whom the outbreak strain of *S.* Typhimurium was isolated in April and May 1999. We asked cases about dairy, meat, and produce consumption and other exposures associated with salmonellosis during the 5 days before onset of diarrhea. We queried controls about the same exposures during the 5-day period preceding onset of illness in the case to whom they were matched.

Traceback and traceforward

To identify potential sources for the outbreak, we reinterviewed ill persons who had eaten statistically implicated foods and the retail suppliers of these foods to determine whether any foods shared a common source (e.g., farm, warehouse, production facility). On the basis of these interviews, we and officials from FDA investigated two sprouters on May 20 and May 25, 1999. We examined records of seed receipt and sprout sales and interviewed the owners of supplying seed companies to confirm shipping records and to establish details concerning sales of one implicated lot of clover seed.

Environmental investigation

Along with officials from the FDA, we observed growing procedures at both sprouters and interviewed employees and owners about disinfection and production methods. We collected sprout samples during production and at retail outlets, seed samples before and after presoaking in chlorine, and environmental swabs. We collected all samples in duplicate and transported them double-bagged in insulated containers on ice. All samples were cultured within 24 hours of collection.

Laboratory investigation

The Colorado Department of Public Health and Environment (CDPHE) serotyped human *Salmonella* isolates and subtyped human and environmental *S.* Typhimurium isolates by PFGE using standard PulseNet methods (10). The sprout, seed, and environmental samples were cultured at CDPHE and CDC using previously described methods (11).

Statistical analysis

We calculated maximum likelihood estimates of matched odds ratios and their exact 95 percent confidence intervals by using Epi Info version 6.04c (12). We performed exact conditional logistic regression analyses using LogXact version 2.1.1 (13). We calculated p values of odds ratios by the chi-square method and applied Fisher's exact test when expected values in any cell were less than five. Matched odds ratios with 95 percent confidence intervals that excluded 1.0 and p values ≤ 0.05 were considered significant.

RESULTS

Case finding and case-control study

Between January and October 1999, the CDPHE laboratory received 201 human *S.* Typhimurium isolates (isolates received for the same time periods in 1996, 1997, and 1998 were 129, 155, and 110, respectively). Of these, 112 (56 percent) had the outbreak PFGE pattern (figure 1). The median age of persons infected with this strain was 27 years (range, <1–87 years), and 58 percent were female. Sixty-one percent of the infections were reported from the state's sparsely populated (<15 percent of the population) Western Slope region, with a notable cluster in one city. Most of the remaining infections occurred in the state's more densely populated Front Range region. No increase in *S.* Typhimurium infections above seasonal baseline was reported from surrounding states.

Between May 11th and 19th, we enrolled 20 cases and 58 controls in our study; all but two cases were matched with three controls. The median age of cases was 29.5 years (range, 8–62 years), and 55 percent were female. Among the 20 cases, 10 (50 percent) reported bloody diarrhea, 19 (95 percent) reported abdominal cramps, 18 (90 percent) reported fever, 16 (80 percent) nausea, 15 (75 percent) headache, and 10 (50 percent) vomiting. The median duration of diarrhea was 8 days (range, 2–21 days), 80 percent took antibiotics, three were hospitalized, and none died.

Cases and controls did not differ significantly according to age, sex, immunosuppressed status, treatment to reduce gastric acidity, or other preexisting conditions and behaviors

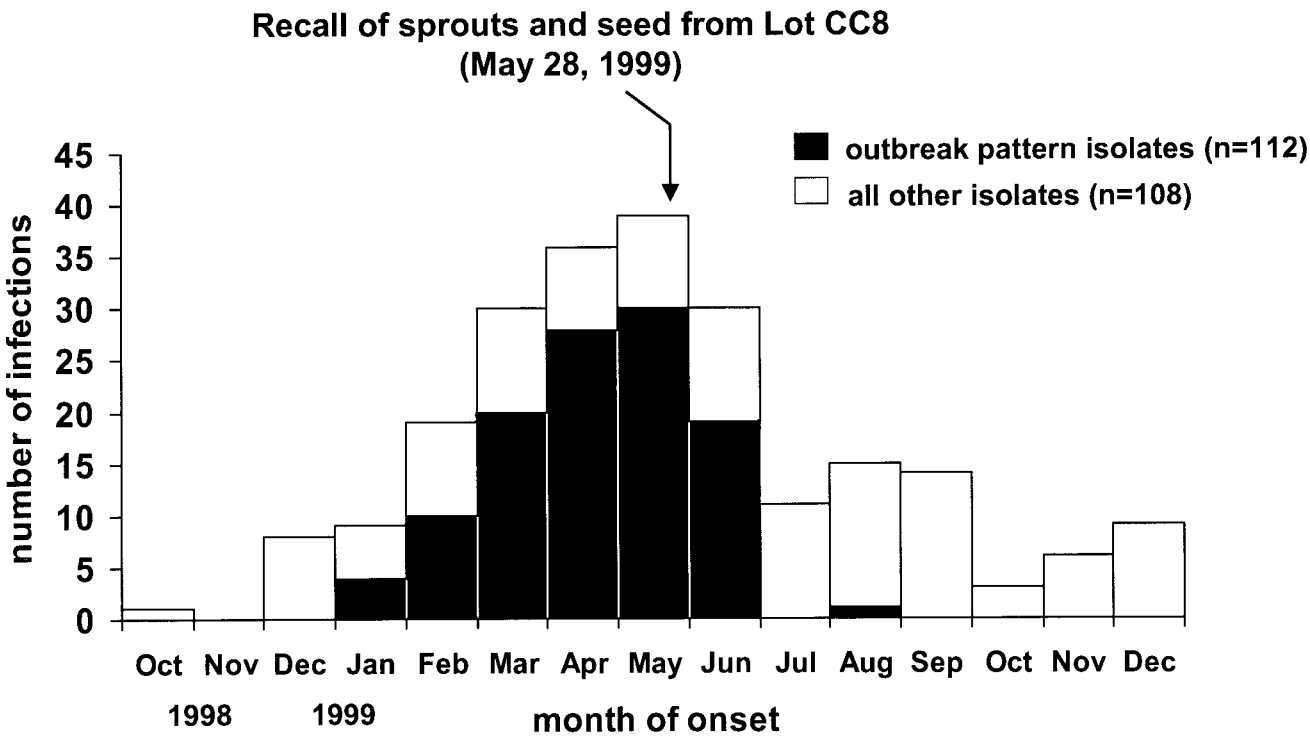

FIGURE 1. Reported *Salmonella* Typhimurium infections in Colorado by month and by PFGE pattern type, 1999.

that could modify risk for salmonellosis (table 1). In univariate analysis, three exposures were statistically associated with risk of illness (table 2). Eighty percent of the cases and 36 percent of the controls ate beefsteak or hothouse-style tomatoes (matched odds ratio (OR) = 5.80, 95 percent confidence interval (CI): 1.9, 21.2). Fifty-two percent of the cases and no controls ate alfalfa-style sprouts (undefined matched OR, 95 percent CI: 6.7, ∞). Sixty-five percent of the cases and 36 percent of the controls ate sliced turkey (matched OR = 2.81, 95 percent CI: 1.0, 8.0). In a multivariate analysis using a model containing tomatoes, sprouts, and sliced turkey, only beefsteak/hothouse-style tomatoes and alfalfa-style sprouts remained independently and significantly associated with illness, producing model-based odds ratios of 11.1 (95 percent CI: 1.4, 457) and 26.8 (95 percent CI: 4.2, ∞), respectively.

TABLE 1. Characteristics of cases and controls, *Salmonella* Typhimurium outbreak, Colorado, 1999

Characteristic	Cases (n = 20) (%)		Controls (n = 58) (%)		p value
Age (median (range))	29.5	(8–62)	31.5	(3–65)	0.63
Female sex	11	(55)	29	(50)	0.70
Vegetarian	0	(0)	0	(0)	1.00
Smoker/chews tobacco	6	(30)	20	(34)	0.54
Exposure to animals in the 5 days prior to illness in the case	3	(15)	8	(14)	0.57
Took antibiotics in the 4 weeks prior to illness in the case	0	(0)	8	(14)	0.18
Took antacids in the 4 weeks prior to illness in the case	6	(30)	17	(29)	0.62
Received immunosuppressive therapy in the 4 weeks prior to illness in the case	0	(0)	1	(2)	0.56
Reported an immunosuppressive medical condition (excluding pregnancy)	2	(10)	5	(9)	0.60
History of stomach surgery	0	(0)	1	(2)	0.56

TABLE 2. Frequency of selected exposures among cases and controls, *Salmonella* Typhimurium outbreak, Colorado, 1999

Exposure	Cases (no. exposed/total no.) (%)		Controls (no. exposed/total no.) (%)		Matched OR*	95% CI*	*p* value
Produce							
Any type of raw tomato	16/20	(80)	27/57	(47)	5.36	1.5, 24.7	0.006
Raw beefsteak/hothouse-style tomatoes	16/20	(80)	21/58	(36)	5.80	1.9, 21.2	0.001
Raw plum/Roma tomatoes	4/20	(20)	9/58	(16)	1.41	0.3, 5.4	0.42
Raw cherry tomatoes	2/20	(10)	7/57	(12)	0.82	0.1, 4.7	0.61
Any raw sprouts	10/20	(50)	2/58	(3)	28.07	4.7, 616	0.00001
Alfalfa-style sprouts	10/19	(52)	0/58	(0)	Undefined	6.7, ∞	<0.00001
Bean sprouts	1/20	(5)	2/58	(3)	1.50	0.1, 19.7	0.58
Any type of lettuce	13/17	(76)	41/57	(76)	0.91	0.2, 4.4	0.59
Iceburg lettuce	13/20	(65)	35/58	(60)	1.21	0.4, 3.7	0.47
Romaine lettuce	5/19	(26)	11/56	(20)	1.42	0.3, 6.1	0.44
Any parsley	2/20	(10)	1/58	(2)	6.00	0.5, 176	0.16
Salsa	11/20	(55)	24/57	(42)	1.76	0.6, 5.6	0.22
Meat							
Sliced turkey (prepack or deli)	13/20	(65)	21/58	(36)	2.81	1.0, 8.0	0.03
Meat from whole turkey or turkey parts	0/20	(0)	7/58	(12)	Undefined	∞, 1.9	0.23
Any hamburger or ground beef	15/20	(75)	44/57	(77)	0.85	0.2, 3.1	0.51
Rare hamburger or ground beef	2/17	(12)	0/58	(0)	Undefined	0.6, ∞	0.10
Beef jerky	3/19	(16)	7/58	(12)	1.63	0.3, 8.3	0.40
Venison or wild game (e.g., elk, moose)	1/19	(5)	8/58	(14)	0.28	0.01, 2.3	0.24
Sausage	8/20	(40)	21/58	(36)	1.23	0.4, 3.7	0.46
Hot dog	4/20	(25)	14/58	(24)	0.70	0.1, 3.0	0.46
Dairy							
Raw or unpasteurized milk or cream	0/20	(0)	0/58	(0)	Undefined		1.00
Cheese from raw/unpasteurized cow's milk	0/20	(0)	0/58	(0)	Undefined		1.00

* OR, odds ratio; CI, confidence interval.

Traceback

The 12 (75 percent) ill persons of 16 available for reinterview who ate beefsteak or hothouse-style tomatoes reported eating tomatoes from eight different retail outlets. Each retail outlet used a different tomato supplier. We believed that it was highly unlikely that eight different suppliers to eight different retailers would have received tomatoes from one common source. The North American regions supplying tomatoes change from January through May; it would require extraordinarily unusual circumstances for one grower to produce tomatoes for multiple Colorado retailers for 5 months. In previously reported outbreaks involving tomatoes (14, 15), illnesses have spanned less than 8 weeks. A large hothouse grower on the Western Slope, which supplied tomatoes to retail outlets where some ill persons had shopped, first began producing tomatoes 2 months after the outbreak started. Other hothouse growers in the region reported selling tomatoes in Colorado and neighboring states, but no unexpected illnesses had been reported outside Colorado.

Among the seven of 10 (70 percent) persons available for reinterview who ate alfalfa-style sprouts, five purchased sprouts from a single supermarket chain, supermarket chain A. Most of supermarket chain A's retail outlets (80 percent, 33 of 41) were located on the Western Slope. Supermarket chain A received the majority of its sprouts from a single supplier, sprouter X, and bought over 95 percent of sprouter X's alfalfa-style product.

Sprouter X produced alfalfa-style sprouts from alfalfa, radish, and clover seeds. In late 1998, supermarket chain A, after learning that alfalfa sprouts had been associated with outbreaks of gastroenteritis, requested that sprouter X cease using alfalfa seed in any products for their stores. Subsequently, sprouter X switched to sprouting mainly clover seeds for supermarket chain A, both alone and in mixtures with radish sprouts. Supermarket chain A carried other brands of sprouts but used sprouter X's clover sprouts exclusively at the deli counters and salad bars in their stores; three of five cases who recalled eating alfalfa-style sprouts from supermarket chain A had obtained them from the salad bar or deli.

Distribution through supermarket chain A could not have accounted for the cases along the Front Range, where roughly one third of the infections were reported. Interviews with four persons infected with the outbreak strain (not included in the case-control study) from three Front Range cities revealed that they had all eaten sprouts at unrelated retail outlets sup-

plied by another sprouter, sprouter Y. Sprouter Y distributed sprouts through a local wholesaler to individual markets and food establishments along the Front Range.

Seed company P was the principal supplier of clover seed to sprouter X and, since December 1998, had been supplying clover seed from one lot, designated lot CC8. Seeds from lot CC8 had been sent to sprouter X in three 1,000-pound (2,202.6-kg) shipments between December 1998 and March 1999. After sprouter X exhausted his supply of lot CC8, he began purchasing clover seed from an unrelated lot supplied by another seed company and first shipped in early May 1999. At the time of our inspection, sprouter X was no longer using lot CC8 seed.

Sprouter Y also used mostly clover seed, also supplied by seed company P, and also from lot CC8. Seed company P was the sole supplier of clover seed to sprouter Y and had sent three separate 2,500-pound (5,506.6-kg) shipments between January and April 1999. Shipping invoices indicated that lot CC8 was the only seed shared by the two sprouters. The seed for lot CC8 originated in Oregon, was bagged for shipment in September or October 1998, and was most likely sold entirely to seed company P. The owner of seed company P reported that among the 13 companies producing sprouts in Colorado during late 1998 and early 1999, he had supplied lot CC8 clover seed only to sprouters X and Y. The date lot CC8 clover seed was first sent to each sprouter corresponded to the subsequent onset of illnesses in the regions that each sprouter supplied (figure 2).

Traceforward

Seed company P sold lot CC8 from November 1998 through May 1999. The entire lot consisted of 383 50-pound (110.1-kg) bags of seed, of which 210 bags (55 percent) were distributed in Colorado, with 60 bags (16 percent) being distributed to sprouter X and 150 bags (39 percent) to sprouter Y. Seven sprouters in the seven other states received less than 1–21 percent of the remainder of lot CC8. None of these states reported notable increases in *S.* Typhimurium infections. Among the non-Coloradan sprouters who received the largest portions of lot CC8, one sprouter in Hawaii who received 80 bags (21 percent) could not confirm having used the implicated seed, and another sprouter in Pennsylvania who received 56 bags (15 percent) had distributed product across multiple states and had been screening his sprout product for bacterial contamination during growth. The remaining five non-Coloradan sprouters each received less than 4 percent of the remainder of lot CC8.

On the basis of the geographic distribution of culture-confirmed infections, we attributed 68 illnesses to sprouter X and 44 illnesses to sprouter Y. Proportionally, four times more illnesses occurred per 50-pound (110.1-kg) bag of seed shipped to sprouter X: 1.13 culture-confirmed illnesses/50-pound bag for sprouter X versus 0.29 culture-confirmed illnesses/50-pound bag for sprouter Y (figure 3).

Active national case finding for the outbreak strain of *S.* Typhimurium identified 21 isolates from two states bordering Colorado; eight isolates were from persons probably exposed to sprouts through supermarket chain A. Eighteen other states reported 63 isolates with the outbreak PFGE pattern, all of which were reported after October 1998, that is, after lot CC8 was available for sale. Exposure to sprouts for these 63 infections was not ascertained. No infections were reported from Pennsylvania or Hawaii.

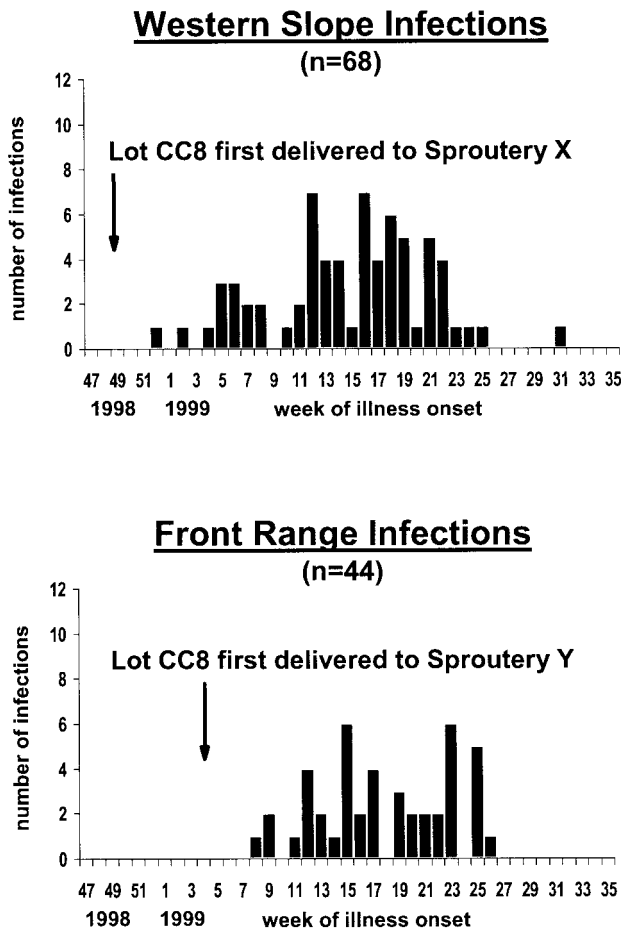

FIGURE 2. Reported *Salmonella* Typhimurium infections with the outbreak pulsed-field gel electrophoresis pattern by week of onset, Western Slope and Front Range, Colorado, 1999.

Environmental investigation

Sprouters X and Y used multiple seed types. At both sprouters, seeds were stored in clean, dry, rodent-free areas. For sprouting, seeds were misted and mixed at 20–22°C in 6-foot (184.2-cm) diameter drums that rotated continuously. For mixed sprouts, such as clover-radish mix, seeds were combined prior to germination.

Sprouter X neither rinsed nor soaked seeds in water or a concentrated chlorine solution before sprouting. In contrast, sprouter Y reported soaking all seeds (with intermittent agitation) for 20 minutes in a 20,000 mg/liter calcium hypochlorite solution and then rinsing the seeds six times in tap water immediately before sprouting. Both sprouters used adequately chlorinated municipal water.

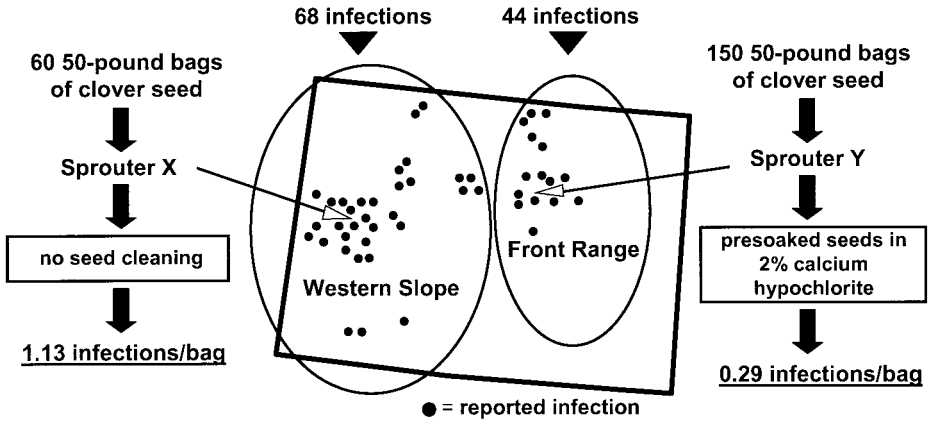

FIGURE 3. Schematic distribution of culture-confirmed *Salmonella* Typhimurium infections with the outbreak pulsed-field gel electrophoresis pattern and attack rates per 50-pound (110.1-kg) bag of clover seed, by region, Colorado, 1999.

Sprouter X rinsed sprouting drums with tap water under normal pressure. Sprouter Y scrubbed each drum with soap, chlorine, and disinfectant and rinsed them with tap water under high pressure. Hygienic practices were otherwise comparable. Neither sprouter had evidence of rodent or insect infestation. No employees reported having a diarrheal illness between January and May 1999.

Laboratory investigation

During 1999, the CDPHE laboratory serotyped 220 *Salmonella* isolates as *S.* Typhimurium. Of these, 112 isolates produced the outbreak strain PFGE pattern. None of the sprout, seed, or environmental samples cultured by the CDPHE and CDC yielded *S.* Typhimurium. However, one environmental swab collected from sprouter Y by the FDA during an inspection coincident with this investigation yielded *S.* Typhimurium. The swab was collected from a cart used to transfer grown sprouts from the rotating bins to a rinsing device and was taken during the processing of clover sprouts grown from lot CC8 seed. The PFGE pattern of this isolate was indistinguishable from the unique outbreak strain pattern.

DISCUSSION

An outbreak of *S.* Typhimurium infections in Colorado resulted from consuming raw sprouted clover seeds. The outbreak was detected through routine serotype-based surveillance enhanced by PFGE. A case-control study identified three exposures significantly associated with illness, but only the strongest association with alfalfa-style sprouts was supported by further epidemiologic investigation.

Many consumers may not recall eating sprouts because, unlike tomatoes and sliced turkey, they are rarely the principal part of a meal, and within composite foods (e.g., deli sandwiches, mixed salads) they may go unnoticed. Previous investigations of sprout-associated outbreaks have found that the number of persons who usually recall eating sprouts is 40 percent or less (16–21). Persons who ate turkey and tomatoes in deli sandwiches (an exposure more common among cases than among controls) may have had unrecognized exposures to sprouts. If exposure to sprouts was misclassified as absent in these settings, multivariate analysis would be expected to inappropriately implicate confounding exposures (e.g., tomatoes).

Traceback demonstrated concordance in time and space between onsets of illness and the delivery of one lot of Oregon clover seed to two Colorado sprouters, which supplied the sprouts eaten by the cases. On March 31, 1999, the CDPHE recalled the implicated sprouts, and seed company P voluntarily recalled and halted further shipment of the source clover seed. Subsequently, the outbreak subsided. Isolation of the outbreak strain of *S.* Typhimurium from an implicated sprouter supported the conclusions of the epidemiologic investigation.

Although reliable data on sprout seed sales for human consumption are lacking, sprouters have anecdotally reported (as in this outbreak) increasingly using clover seed for the production of alfalfa-style sprouts. They perceive clover seed to be safer because the smoother seed coat may theoretically be less hospitable to bacterial contamination and more amenable to chemical disinfection. Earlier sprout-related outbreaks have implicated clover sprouts when these sprouts were eaten in mixtures with other sprouts (22). This investigation demonstrated that clover sprouts alone can serve as a vehicle for transmitting enteric infection. Additional outbreaks later in 1999 confirmed the risk of illness associated with eating raw clover sprouts alone (23).

If sprouter Y consistently disinfected seeds as observed during our investigation, then this outbreak also documented that treating seeds with a 20,000 mg/liter calcium hypochlorite solution substantially reduces, but does not eliminate, the risk of illness from sprouts. Recent FDA laboratory studies with naturally contaminated seeds (24) confirm that this treatment reduces, but does not eliminate, pathogenic bacteria. These experiments were conducted using seeds from a sprout-associated *Salmonella* outbreak that occurred later in 1999 (25), in which the sprouter had

routinely pretreated alfalfa seed with a 20,000 mg/liter calcium hypochlorite solution as recommended (4). Assuming that contamination of the seed implicated in the Colorado outbreak was uniform, that the seed was not subjected to environmental contamination or rodent infestation, that the sprouters grew and sold product in proportions equal to the amount of the seed they each received, and that all other biases in packaging, shipment, and storage of the seeds were equally distributed, the different attack rates associated with the two sprouters reflect differences in production. Although the production practices of sprouter X were comparatively less hygienic, there is no evidence that the level of hygiene was inadequate or permitted cross-contamination. The most notable difference was that sprouter Y soaked seeds in a concentrated calcium hypochlorite solution before sprouting them; sprouter X did not.

No *S.* Typhimurium was isolated from sprouter X's facility or products. Sprouter X ceased using clover seed from lot CC8 a few weeks before the unannounced inspection, reducing the likelihood that bacteria from these seeds would be present at that facility when samples were collected. Sprouter Y was using lot CC8 when environmental samples were collected.

In most other sprout-related outbreaks, infections have been ascribed to seeds contaminated with pathogenic bacteria. *Salmonella* experimentally inoculated on alfalfa seed increases 3–4 logs during sprouting (26). During growth, bacteria may become enmeshed in the tissues of the growing sprouts (27), where they cannot be removed by methods routinely used by consumers to clean raw vegetables (e.g., washing, soaking, scrubbing). *E. coli* O157 has been cultured from the inner tissues of sprouts grown from seed after bacteria coating the outside of the sprouts were removed by disinfecting rinses (27). Heating sprouts has been recommended to make them safe to eat but may render the product unappealing. Irradiation, which was recently approved for treating sprout seeds (28), may also provide a viable option for disinfecting grown sprouts (29).

No seed samples yielded the outbreak strain of *S.* Typhimurium. In most previously reported, sprout-associated outbreak investigations, the outbreak bacterial strain was not isolated from implicated seeds despite abundant epidemiologic evidence that seeds were the source. Recovery of bacteria may be complicated by uneven and low levels of seed contamination and the limited number of samples tested. *Salmonella* has been recovered from sprouts grown under aseptic conditions using seeds from which bacteria could not be isolated before sprouting (30). In two sprout-associated *Salmonella* outbreaks, the outbreak strain of bacteria was successfully recovered from implicated seeds (20, 21).

How seeds become contaminated is not fully understood. Sprouts for human consumption, including clover and alfalfa sprouts, come from seed grown for animal feed and are considered a raw agricultural commodity. Improperly composted manure containing pathogenic bacteria might be used to fertilize fields. Domesticated and wild animals may graze in and defecate on seed fields. Small amounts of bacteria left behind in devices used for harvesting and processing may cross-contaminate seeds. Rodents and birds carrying bacteria can infest storage facilities. In this outbreak, the FDA determined that one seed packer in Oregon produced the implicated lot by using mixed clover seed from more than 20 growers. Records of which growers' seed contributed to which lots were not maintained; further traceback to one field or farm was not feasible.

Circumstances in Colorado uniquely converged to facilitate detection of this outbreak. The majority of the implicated seed was shipped to only two sprouters in the state, who, in turn, distributed product almost exclusively within the state's boundaries, including to areas of lower population density. In other states to which the implicated seed was shipped, most sprouters received lesser quantities. In states to which comparable amounts of the seed were sent, either sprouting of the seed could not be confirmed, sprouts were distributed over multiple states within areas of greater population density, or the sprouter used methods to detect the presence of potentially pathogenic bacteria during sprout growth before the product was sold to consumers. Other possible explanations for the lack of illnesses detected outside Colorado include nonuniform contamination of the implicated seed, variable sensitivity of the surveillance systems in other jurisdictions, and more rigorous adherence by other sprouters to recommended seed disinfection protocols and hygiene.

PFGE subtyping facilitated early recognition of this outbreak and identified persons with illnesses most likely to be epidemiologically related. *S.* Typhimurium is the most common *Salmonella* serotype reported in the United States, accounting for 25 percent of *Salmonella* isolates reported to the CDC and the CDPHE in 1999 (31). Limiting our case definition to ill persons whose *S.* Typhimurium isolates produced the outbreak PFGE pattern increased the power of the case-control method.

One month after the investigation, the FDA released a consumer advisory expanding the earlier advisory that applied only to alfalfa sprouts. Citing the findings from this outbreak (32), the revised advisory noted that 1) all raw sprouts pose a potential risk of infection to all persons, 2) despite growers properly implementing approved methods to disinfect sprout seeds, illnesses continue to be associated with eating raw sprouts, and 3) consumers should cook sprouts to make them safe to eat. Three months later, the FDA released its first guidance for industry to improve the safety of raw sprouts (9). In addition to reiterating good manufacturing practices and specifying the means for decontaminating sprout seed with chlorine solutions, this document recommended that sprouters test spent sprout irrigation water to identify bacterial contamination before releasing their product into commerce.

The number of outbreaks caused by raw sprouts may appear to be small (4), but sprouts are also consumed infrequently compared with other fruits and vegetables (2, 3). Considering additionally that sprouts are produced under conditions that favor rapid bacterial growth, the risk per serving of untreated raw sprouts to consumers may be substantially greater than that of other foods. Persons wishing to avoid any potential exposures to *Salmonella* or *E. coli* O157:H7 infec-

tions or those who are at risk of serious complications resulting from these bacterial infections (e.g., young children, elderly adults, and persons with weakened immune systems) should not eat raw sprouts. If consumers choose to eat them, the FDA recommends that sprouts be cooked (32). Better methods to disinfect seeds and sprouts (including irradiation and other chemicals) and to detect contaminated product during growth deserve investigation. Designating seed fields to produce sprouts for human consumption only, fertilizing seed fields only with properly composted manure or inorganic fertilizers, and harvesting and processing seed for human consumption separately from seed for animal feed are interventions that merit consideration.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Elizabeth Hannah for her generous assistance in conducting interviews and facilitating site visits, to Kelly Kamm at the CDPHE and Nancy Puhr at the CDC for their assistance in processing specimens and serotyping isolates, to the staff of the FDA's Center for Food Safety and Applied Nutrition for their editorial assistance and insights regarding sprout production, to the FDA's Denver District Office for their generous laboratory contribution and assistance with inspections, to the FDA field staff who assisted with traceback and traceforward activities outside of Denver, and to the staff of the Mesa County Health Department for their dedication investigating this outbreak and for their hospitality.

REFERENCES

1. Taormina PJ, Beuchat LR, Slutsker L. Infections associated with eating seed sprouts; an international concern. Emerg Infect Dis 1999;5:626–34.
2. Foodborne Diseases Active Surveillance Network (FoodNet). Population survey atlas of exposures: 1997–1998. Atlanta, GA: Centers for Disease Control and Prevention, 1998. (http://www.cdc.gov/ncidod/dbmd/foodnet/surveys/Pop_surv.htm).
3. Foodborne Diseases Active Surveillance Network (FoodNet). Population survey atlas of exposures: 1998–1999. Atlanta, GA: Centers for Disease Control and Prevention, 1999. (http://www.cdc.gov/ncidod/dbmd/foodnet/surveys/Pop_surv.htm).
4. National Advisory Committee on Microbiological Criteria for Foods. Microbiological safety evaluations and recommendations on sprouted seed. Int J Food Microbiol 1999;52:123–53.
5. Food and Drug Administration. Interim advisory on alfalfa sprouts (T98–47). Washington, DC: Food and Drug Administration, 1998. (www.cfsan.fda.gov/~lrd/tpalfalf.html).
6. Beuchat LR. Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. Int J Food Microbiol 1997;34:329–33.
7. Taormina PJ, Beuchat LR. Behavior of enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa sprouts during the sprouting process as influenced by treatments with various chemicals. J Food Prot 1999;62:850–6.
8. Taormina PJ, Beuchat LR. Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. J Food Prot 1999;62:318–24.
9. Guidance for industry: reducing microbial food safety hazards for sprouted seeds. Center for Food Safety and Applied Nutrition. Washington, DC: Food and Drug Administration, 1999. (http://vm.cfsan.fda.gov/~dms/sprougd1.html).
10. Swaminathan B, Barrett TJ. A national molecular subtyping network for food-borne bacterial disease surveillance in the United States. In: Nachamkin I, Blaser MJ, eds. *Campylobacter.* Washington, DC: American Society for Microbiology, 2000:529–35.
11. Cook K, Dobbs TE, Hlady WG, et al. Outbreak of *Salmonella* serotype Hartford infections associated with unpasteurized orange juice. JAMA 1998;280:1504–9.
12. Dean AG, Arner TG, Sangam S, et al. Epi Info version 6: a word processing, database, and statistics program for public health on IBM-compatible microcomputers. Atlanta, GA: Centers for Disease Control and Prevention, 2000.
13. LogXact for Windows, A software package for exact logistic regression. Cambridge, MA: Cytel Software Corp, 1996.
14. Hedberg CW, Angulo FJ, White KE, et al. Outbreaks of salmonellosis associated with eating uncooked tomatoes: implications for public health. Epidemiol Infect 1999;122:385–93.
15. Cummings K, Barrett E, Mohle-Boetani J, et al. A multistate outbreak of *Salmonella baildon* associated with domestic tomatoes. Emerg Infect Dis (In press).
16. Mouzin E, Werner SB, Bryant RG, et al. When a health food becomes a hazard: a large outbreak of salmonellosis associated with alfalfa sprouts—California. Presented at the 46th Annual Epidemic Intelligence Service (EIS) Conference. Atlanta, GA: Centers for Disease Control and Prevention, 1997:15.
17. Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts—Michigan and Virginia, June-July 1997. MMWR Morb Mortal Wkly Rep 1997;46:741–4.
18. Mahon BE, Ponka A, Hall WN, et al. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. J Infect Dis 1997;175:876–82.
19. Wegener HC, Baggesen DL, Neimann J, et al. An outbreak of human salmonellosis in Denmark caused by alfalfa sprouts. In: Colin P, Le Goux JM, Clement G, eds. *Salmonella* and salmonellosis. Ploufragan, France: Zoopole, 1997:587–9.
20. Glynn MK, Patrick S, Wuhib T, et al. When health food isn't so healthy—an outbreak of *Salmonella* serotypes Anatum and Infantis associated with eating contaminated sprouts, Kansas and Missouri, 1997. Presented at the 47th Annual Epidemic Intelligence Service (EIS) Conference. Atlanta, GA: Centers for Disease Control and Prevention, 1998.
21. Van Beneden CA, Keene WE, Strang RA, et al. Multinational outbreak of *Salmonella enterica* serotype Newport infections due to contaminated alfalfa sprouts. JAMA 1999;281:158–62.
22. Moehle-Boetani CJ, Farrar JF, Werner SB, et al. *Escherichia coli* O157 and *Salmonella* infections associated with sprouts in California, 1996–1998. Ann Intern Med 2001;135:239–47.
23. California Department of Health Services. State health department warns consumers about sprouts. Sacramento, CA: California Department of Health Services, 1999. (http://www.dhs.cahwnet.gov/opa/prssrels/1999/28–99.htm).
24. Stewart DS, Reineke KF, Ulaszek JM, et al. Growth of *Salmonella* during sprouting of alfalfa seeds associated with salmonellosis outbreaks. J Food Prot 2001;64:618–22.
25. Proctor ME, Hamacher M, Tortorello ML, et al. Multistate outbreak of *Salmonella muenchen* infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. Presented at the International Conference on Emerging Infectious Diseases, 2000. Atlanta, GA: Centers for Disease Control and Prevention, 2000:98.
26. Andrews WH, Mislivec PB, Wilson CR, et al. Microbial hazards associated with bean sprouting. J Assoc Off Anal Chem 1982;65:241–8.
27. Itoh Y, Sugita-Konishi Y, Kasuga F, et al. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. Appl Environ Microbiol 1998;64:1532–5.
28. Federal Register. Irradiation in production, processing, and

handling of food—seeds for sprouting; safe use of ionizing radiation for microbial pathogens. Fed Regist 2000:64605–7.
29. Rajkowski KT, Thayer DW. Reduction of *Salmonella* spp. and strains of *Escherichia coli* O157:H7 by gamma radiation of inoculated sprouts. J Food Prot 2000;63:871–5.
30. Splittestoesser DF, Queale DT, Andalaro BW. The microbiology of vegetable sprouts during commercial production. J Food Safety 1983;5:79–86.
31. Centers for Disease Control and Prevention. *Salmonella* surveillance: annual tabulation summary, 1999. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, 2000.
32. Food and Drug Administration. Consumers advised of risks associated with raw sprouts (P99–13). Washington, DC: Food and Drug Administration, 1999. (http://www.fda.gov/bbs/topics/NEWS/NEW00684.html).