

Multistate Outbreak of *Salmonella* Serovar Muenchen Infections Associated with Alfalfa Sprouts Grown from Seeds Pretreated with Calcium Hypochlorite

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During September 1999, a multistate outbreak of *Salmonella* serovar Muenchen infection associated with eating raw alfalfa sprouts was identified in Wisconsin. Despite use of a calcium hypochlorite sanitizing procedure to pretreat seeds before sprouting, at least 157 outbreak-related illnesses were identified in seven states having sprouters who received alfalfa seed from a specific lot. The continued occurrence of sprout-related outbreaks despite presprouting disinfection supports the concern that no available treatment will eliminate pathogens from seeds before sprouting and reinforces the need for additional safeguards to protect the public. A lack of consumer knowledge regarding exposure to sprouts documented in this investigation suggests that more-targeted outreach to high-risk individuals may be needed to reduce their risk.

Consumption of raw sprouts has emerged as an important risk factor associated with the occurrence of food-borne illness. In the United States, at least 12 reported sprout-related disease outbreaks involving a total of more than 1,500 cases have been reported since 1995. Sprout-related illness has involved infection with 10 different serogroups of *Salmonella* (12, 13, 15–17, 20; E. Mouzin et al., Prog. Abstr. 46th Annu. Epidemic Intell. Service Conf., p. 15, 1997; P. Buck et al., Prog. Abstr. 47th Annu. Epidemic Intell. Service Conf., p. 39, 1998; M. K. Glynn et al., Prog. Abstr. 47th Annu. Epidemic Intell. Service Conf., p. 16, 1998) and with *Escherichia coli* O157:H7 (5, 9, 14). Most sprout-related outbreaks have been associated with consumption of alfalfa or clover sprouts. Among 12 outbreaks reported since 1995, 9 involved inherently contaminated seeds (9, 12–17, 20; Buck et al., Prog. Abstr. 47th Annu. Epidemic Intell. Service Conf.; Glynn et al., Prog. Abstr. 47th Annu. Epidemic Intell. Service Conf.) and investigations of 3 outbreaks could not distinguish whether the outbreaks involved contaminated seeds or sprouter error (5, 12; Mouzin et al., Prog. Abstr. 46th Annu. Epidemic Intell. Service Conf.).

This report describes a nationwide outbreak detected in Wisconsin during September 1999 which was associated with eating raw alfalfa sprouts that were grown from seeds that had received recommended calcium hypochlorite sanitizing before sprouting. A predominantly adult female profile among case patients and the lack of clustering of cases by household, defined social gatherings, or restaurant visits were observed in this and in previously reported sprout-related outbreaks (13, 20).

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MATERIALS AND METHODS

Background. Sixteen isolates of tartrate-negative *Salmonella* serovar Muenchen were identified between 29 August and 27 September in the Bacteriology Laboratory, Wisconsin State Laboratory of Hygiene (WSLH); the isolates were from residents of three Wisconsin counties with illness onsets between 20 August and 18 September 1999. The usual number of *Salmonella* serovar Muenchen isolates reported in Wisconsin in a given year is nine. The WSLH notified the Communicable Disease Epidemiology Section, Wisconsin Division of Public Health (WDPH), on 28 September.

Case finding. On 29 September, Wisconsin clinical microbiology laboratories were notified by electronic mail and asked to report and forward until the end of the year all *Salmonella* serovar Muenchen isolates to the WSLH for confirmation and for pulsed-field gel electrophoresis (PFGE) analysis. Wisconsin's outbreak cluster was posted on the Centers for Disease Control and Prevention's (CDC's) ListServe on 30 September, and the *Salmonella* serovar Muenchen PFGE outbreak pattern was posted on CDC's PulseNet on 4 October.

Case control study. A case control study was conducted to identify potential exposures associated with illness. Case patients for the case control study included 16 individuals who experienced onset of illness during 29 August to 27 September and who had been diagnosed with laboratory-confirmed tartrate-negative *Salmonella* serovar Muenchen infection by 30 September, the day before the case control study was initiated. Controls were located by sequential-digit dialing based on the telephone number of the case patient to whom they were being matched. Two controls were located for each of the 16 cases and were matched by gender and age group (≤ 10 years old, ± 2 years; 11 to 20 years old, ± 3 years; >21 years old, ± 5 years). Case patients and controls were interviewed during 1 to 3 October using the 10-page Minnesota Standard Food-Borne Disease Exposure Questionnaire (K. Smith, personal communication). If the case patient or control was less than 18 years of age, a parent or guardian was asked to provide answers to the questionnaire.

Outbreak control measures. Following the availability of the results of the case control study on 3 October, a press release advising against selling, serving, or eating sprouts of any type in the three-county area where the first cases were identified was issued jointly on 5 October by the Wisconsin Department of Agriculture, Trade, and Consumer Protection (WDATCP) and WDPH.

Traceback activities and environmental investigation. On 6 October, WDATCP and the local health department staff began collecting intact (unopened) packages of sprouts from grocery stores and restaurants within their

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jurisdiction that had been identified by case patients as a potential source of any sprouts they may have consumed. Sprout exposure data obtained from 96-h food histories of the first 16 case patients were traced back to Wisconsin sprout distributors A, B, and C and to Wisconsin sprouters A and B.

The WDATCP staff collected sprout, environmental, and water samples from Wisconsin sprouters A and B between 9 and 12 October. Collected sprout samples included refrigerated sprout packages at both sprout facilities. Environmental samples included sterile saline swabs of work areas and sprouting equipment surfaces, production and packing materials, floor drains, and air vents. Water samples included collections in sterile containers of tap water from all available faucets in the sprout production and storage areas and well water at the sprouter B facility. All specimens were transported to the WDATCP laboratory for testing.

Sprout disinfection procedure. Wisconsin sprouters A and B routinely disinfected sprouts according to a then-current Food and Drug Administration (FDA)-recommended procedure. Specifically, after a prewash to remove visible dirt, 5 lb of seed was treated for 15 min in 1 gal of 2% calcium hypochlorite, drained, and rinsed again with potable water. Disinfected, washed seeds were either sprouted for 3 to 7 days in rigid containers with intermittent spraying or were sprouted in rotating drums for 3 days, with eventual sale in 5-lb trays or plastic bags.

Pathogen rapid-testing experiments conducted by FDA at the time of outbreak. Coincidentally, during the spring and summer of 1999, an FDA researcher and one of the authors (M.L.T.) had been conducting a series of experiments at an FDA laboratory to study methods for rapid testing for pathogens in sprouts and sprout irrigation water. Aliquots of seed from alfalfa seed lot COA98 were being inoculated with four serotypes of *Salmonella*, including serovars St. Paul, Tennessee, Cubana, and Havana. Noninoculated seeds from lot COA98 were used for control purposes. Alfalfa seeds were obtained from International Specialty Supply (Cookeville, Tenn.) and sprouted in glass jars. Sprouts were rinsed with sterile water, and samples of the sprouts and irrigation water were inoculated with various levels of three serovars of *Salmonella* for evaluation of rapid-assay methods. Inoculated samples and uninoculated controls were added to enrichment media according to the rapid-assay protocols, viz., the *Salmonella* Assurance Gold enzyme immunoassay and the *Salmonella* visual immunoprecipitate assay (Biocontrol Systems, Inc., Bellview, Wash.) (2). Regardless of the rapid-assay results, enrichments were streaked onto selective agars for isolation and confirmation of *Salmonella* colonies according to standard methods (1).

Laboratory methods. The WDATCP laboratory obtained and followed the CDC procedure for isolation of *Salmonella* from food. Specifically, 25 g of sprouts was placed in 225 ml of tetrathionate (TET) broth with brilliant green (BG) dye. After incubation for 18 to 24 h at 37°C, an aliquot was streaked on BG and Hektoen enteric (HE) agar plates and also subcultured in additional TET media for another 18 to 24 h at 42°C before being streaked on a second set of BG and HE agar plates for observation of colony development. The same procedure for isolation of *Salmonella* from water and environmental swabs was performed by substituting 25 ml of water or the sterile swab for the initial 25 g inoculated into the 225 ml of TET medium.

PFGE analysis. PFGE was conducted at the WSLH on human and product *Salmonella* serovar Muenchen isolates using standard CDC methods (8). The isolates were digested with either *Xba*I or *Bln*I in different gel runs.

Statistical analysis. Case control data analysis was conducted using the Mantel-Haenszel matched chi square for dichotomous variables with EpiInfo (version 6.0; CDC, Atlanta, Ga). Ninety-five percent confidence intervals for odds ratios were calculated using the method of Cornfield; probability (*P*) values were calculated using Fisher's two-tailed exact test.

RESULTS

Demographic and clinical characteristics. Among the 62 Wisconsin case patients, 42 (68%) were female, the median age was 35 years (range, 13 to 85 years), six (10%) were hospitalized, and no deaths occurred. Chief signs and symptoms reported by the 62 case patients included diarrhea (97%), abdominal cramps (84%), fatigue (81%), chills (68%), headache (68%), fever (66%), nausea (65%), body aches (61%), and vomiting (37%). Three (5%) Wisconsin case patients experienced urinary tract infections (UTI) with *Salmonella* serovar Muenchen isolated from urine. Two of these three case

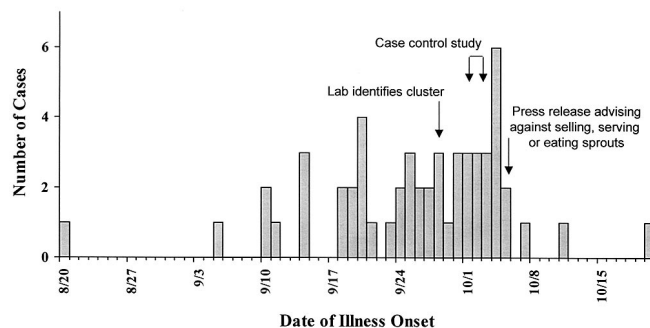


FIG. 1. Laboratory-confirmed cases ($n = 62$) of *Salmonella* serovar Muenchen infection with indistinguishable PFGE patterns by date of onset in Wisconsin from August to October 1999.

patients with UTI did not report antecedent or concurrent diarrheal illness.

Case finding. The day after the *Salmonella* serovar Muenchen PFGE pattern was posted on CDC's PulseNet, two states reported matches to the outbreak pattern. Ultimately, 157 outbreak-related cases were identified, including 95 in six states other than Wisconsin (California, 23; Idaho, 11; Michigan, 35; Missouri, 19; Nevada, 5; Washington, 2). Dates of illness for the 62 Wisconsin case patients were between 20 August and 20 October (Fig. 1), and onsets of non-Wisconsin cases occurred between 8 July and 29 November 1999.

Case control study. The case control study identified sprouts as the implicated vehicle with a matched odds ratio of 28 (95% confidence interval, 1.8 to 956). Some Wisconsin case patients recalled consuming some type of sprouts in the 7 days before illness onset, but not all were able to identify the specific type of sprouts eaten. Among the 32 case patients who were specifically asked about sprout consumption during the 7 days before illness, 10 said they "may have," 10 said they were "unsure," and 12 said "no, I never eat sprouts." Of those who did not specifically recall eating sprouts and who provided a 96-h food history, 55% (10 of 18) ate at a potential exposure site (one that had received the implicated lot of alfalfa sprouts as determined by the traceback investigation) and reported having eaten a food item that routinely includes sprouts at that establishment. These individuals were not aware that they had consumed sprouts.

Outbreak control measures. The Wisconsin press release apparently had a significant impact in preventing additional illness. Among the 62 Wisconsin outbreak-related cases occurring between 20 August and 20 October, only 3 had onsets of illness following the press release (Fig. 1). Following traceback activities, an FDA class I recall implemented between 22 October and 31 October 1999 involved 32,900 lb of alfalfa seeds comprising the remainder of lot COA98.

Environmental investigation. Testing of more than 100 sprout, environmental, and water samples at Wisconsin sprouters A and B did not produce any bacterial isolates. Seven (15%) of 47 intact (unopened) packages of sprouts retrieved from specific grocery stores or restaurants named by case patients were positive for *Salmonella*; 3 packages of alfalfa sprouts were positive for *Salmonella* (IIIa) serovar Arizona

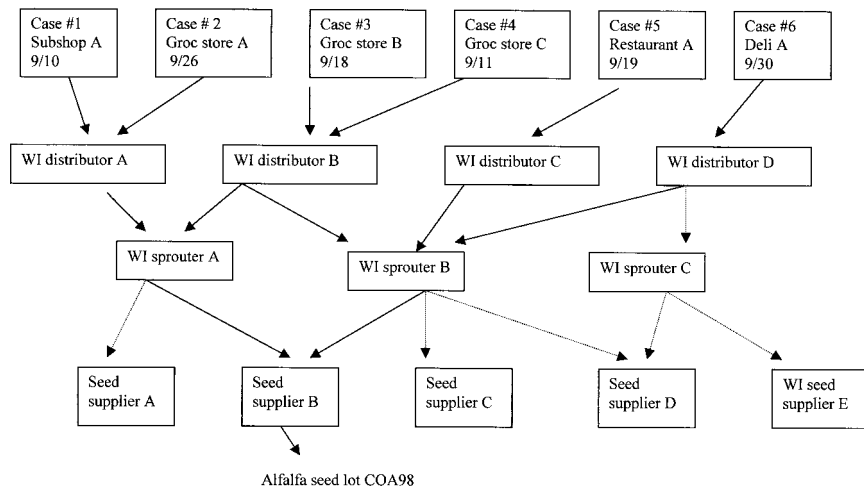


FIG. 2. Traceback of alfalfa sprouts from case patient exposure site through Wisconsin sprout distributors, sprouters, and seed suppliers to a specific seed lot.

bacteria, and 4 packages of alfalfa sprouts were positive for tartrate-negative *Salmonella* serovar Muenchen bacteria.

Traceback procedure. Among 30 case patients who recalled consuming sprouts during the 7 days before illness, 10 ate sprouts on more than one occasion or from more than one site. The other 20 case patients had a single or unique sprout exposure involving 10 different restaurants, 4 grocery stores, and 4 deli or sub shops. Data regarding these 20 unique exposures were used in conducting a sprout traceback. For purposes of simplicity, the traceback schematic illustrated in Fig. 2 includes only 6 case patients but is representative of all 20 case patients with a unique sprout exposure. Retail establishment invoices relevant to all 20 cases with a unique sprout exposure were traced backward to specific Wisconsin sprout distributors. Sprout distributor date-specific invoices which corresponded with the case patient exposure dates were further traced back to two Wisconsin sprouters, both of whom had obtained sprout seeds from seed supplier B. Detailed information regarding specific seed lot sprouting dates and the linkage of these sprouting dates with distribution dates for both Wisconsin sprouters were evaluated. The only seed lot which seed supplier B had shipped to both Wisconsin sprouters that matched up with data from the four Wisconsin sprout distributors and with Wisconsin case consumption dates was alfalfa seed lot COA98.

Seeds in a typical alfalfa seed lot are grown on different farms and then mixed. Seed lot COA98 comprised seeds from 17 farms near the Oklahoma-Texas border and was blended by a seed producer in Oklahoma and distributed by seed supplier B in Tennessee to 33 sprouters in 10 states, including two in Wisconsin. From individual sprouters, alfalfa sprouts were distributed throughout Wisconsin to various retail establishments, grocery stores, restaurants, and cooperatives.

Isolation of outbreak strain from seed lot during sprouting. During the spring and summer of 1999, an FDA researcher and one of the authors (M.L.T.) unexpectedly isolated tartrate-negative *Salmonella* serovar Muenchen from the irrigation water samples of both inoculated and control (uninoculated)

sprouting alfalfa seeds. Aliquots of alfalfa seed lot number COA98 had been experimentally inoculated with *Salmonella* serovar St. Paul, *Salmonella* serovar Tennessee, *Salmonella* serovar Cubana, and *Salmonella* serovar Havana during rapid-testing studies. The researcher contacted the WDPH when she became aware of the Wisconsin *Salmonella* serovar Muenchen sprout outbreak investigation. The alfalfa seed lot number associated with the Wisconsin investigation had not been mentioned in the initial press release. Five *Salmonella* serovar Muenchen isolates from the irrigation water samples were provided by the FDA to the WSLH, where the PFGE pattern was compared with the Wisconsin outbreak investigation *Salmonella* serovar Muenchen PFGE pattern.

Molecular analysis of isolates. *Salmonella* serovar Muenchen isolates from the following individuals or sources were all indistinguishable by PFGE using two different enzymes: 62 Wisconsin case patients, 95 non-Wisconsin case patients residing in six other states receiving alfalfa seed lot COA98, four intact packages of alfalfa sprouts grown from alfalfa seed lot COA98 and obtained from Wisconsin stores or restaurants, and the five isolates from the samples of irrigation water of sprouting seed lot COA98 provided by the FDA laboratory.

DISCUSSION

We conclude that an inherently contaminated alfalfa sprout seed lot was distributed to 33 sprouters in 10 states and resulted in at least 157 *Salmonella* serovar Muenchen outbreak-related illnesses in 7 states. The likely range in number of cases associated with this outbreak was probably 3,500 to 16,200 based on rates of underreporting defined in other *Salmonella* outbreaks (6). Evidence for a contaminated seed lot as opposed to individual sprouter-associated contamination is supported by temporal and geographic clustering of cases associated with multiple sprouting facilities in multiple states receiving the implicated lot and by indistinguishable PFGE patterns of *Salmonella* serovar Muenchen among human iso-

lates from residents of seven states, product isolates from retail sprout packages traced back to lot COA98, and isolates from the irrigation water of the implicated seed lot during sprouting. Distribution of a contaminated seed lot to multiple growers geographically corresponding to the distribution of cases in a nationwide outbreak has been reported (13, 20).

Previously reported sprout-related outbreaks did not provide conclusive documentation that the sprouters had consistently followed recommended presprouting disinfection methods. This is the first reported outbreak with specific documentation (signed FDA affidavit) that an outbreak occurred despite sprout growers (in Wisconsin) having soaked alfalfa seed in 20,000-ppm chlorine solution for 15 min prior to sprouting. It reinforces the fact that currently recommended disinfection methods alone will not prevent continuing sprout outbreaks and reinforces the need for testing of irrigation water as described in the guideline.

Growing, harvesting, processing, mixing, and shipping of seed, followed by sprouting and distribution of finished product provide multiple points where pathogens can be introduced or amplified before reaching the consumer. Viable pathogens can persist not only on the surface but also in the inner tissue and stomata of cotyledons of sprouts experimentally contaminated with bacteria (11). Low numbers of culturable *Salmonella* may not be detected in seed even with methods that provide the greatest potential for detection and recovery (10). While several chemical treatments can reduce *Salmonella* populations up to 3.2 log₁₀ CFU/g on alfalfa seeds, as analyzed by direct plating, no chemical treatment will eliminate the pathogen, as evidenced by detection in enrichment samples (21). Treatment of seeds with a concentration of calcium hypochlorite as high as 2% does not prevent outgrowth of *Salmonella* from naturally contaminated seeds (18). Once present, high humidity and temperatures in the 21 to 25°C range favor amplification of bacteria in or on the seed during germination and sprouting (19). Previous sprout-related outbreaks have established that pathogens can exceed 10⁷ CFU per g of sprouts without adversely affecting the appearance of sprouts and that treating seeds and sprouts with chlorinated water or other disinfectants fails to eliminate pathogens (3, 4). Testing for pathogens following sprouting of seed has been suggested.

An FDA guidance document to improve the safety of raw sprouts was published in the Federal Register on 27 October 1999 (7). In addition to good agricultural practices, the document also recommends that seed disinfection treatments be combined with microbial testing of spent irrigation water from each batch of sprouts for both *Salmonella* and *E. coli* O157:H7. Because testing for pathogens can be done at 48 h into what is generally a 3- to 10-day growing period, producers can obtain results before shipping the product without losing product shelf life and before sprouts enter the food supply.

Until routine testing of spent irrigation water from each batch of sprouts is universally implemented, sprouts, when served raw, remain a potential public health risk, especially to those with a compromised immune system. The only effective way to eliminate risk of food-borne illness from raw sprouts is to avoid eating them. In particular, persons at high risk for severe complications of infection with *Salmonella* and *E. coli* O157:H7, such as the elderly, children, and those with com-

promised immune systems, have been cautioned not to eat raw sprouts (19).

A disturbing finding of this investigation is that people may not be aware that they are consuming sprouts in their food. Even if individuals are trying to reduce their risk by avoiding consumption of sprouts, foods may be served with sprouts without the knowledge of the consumer. Only 48% (30 of 62) of Wisconsin's laboratory-confirmed outbreak-related case patients knowingly chose to eat sprouts during the week before illness. Among the other 32 Wisconsin outbreak case patients, who did not knowingly consume sprouts in the week before illness onset, 38% (12 of 32) would not have eaten sprouts if given the choice. While it is possible that cross contamination of other foods with *Salmonella*-infected sprouts or poor hygienic practices at some restaurants may have been responsible for exposure of individuals who had no recall of sprout consumption, it is also possible that many of these individuals may have unknowingly eaten contaminated sprouts in sandwiches or in salads that they ordered. Restaurants and sandwich shops that routinely include sprouts in salads and sandwiches should ensure that sprouts are prominently listed as components of that food item or allow the customer to specifically choose this ingredient if they order that food item. Because individuals must make informed decisions about food choices, given what we know about the potential public health risk of consuming raw sprouts, food establishments have a shared responsibility to inform consumers that a food item contains sprouts.

A press release warning Wisconsin residents not to sell, serve, or eat sprouts until the specific sprout type associated with the outbreak was identified had a significant impact in preventing additional Wisconsin cases. At the time of the press release, the complex traceback had just begun and alfalfa sprouts from contaminated seeds had not yet been pulled from the shelves and were still available in retail establishments. The traceback investigation took about 1 month to complete and resulted in 32,900 lb of remaining alfalfa seed lot COA98 being recalled nationwide. Outbreak traceback investigations are labor-intensive and need to be timely to have an impact on reducing disease morbidity. However, once a specific seed lot is identified as contaminated and recalled, the risk of other outbreaks is not necessarily eliminated. Because seed lots routinely consist of a mixture of seeds from many seed-supplying farms, traceback and testing of "library" samples of all individual components of an implicated seed lot are essential if the individual farm contributing contaminated seeds is to be identified and appropriate measures taken to eliminate or minimize risk of contamination of future seed lots.

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