Alfalfa Sprouts and *Salmonella* Kottbus Infection: A Multistate Outbreak following Inadequate Seed Disinfection with Heat and Chlorine†

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ABSTRACT

Raw sprouts have been implicated in a number of foodborne disease outbreaks. Because contaminated seeds are usually responsible, many sprout producers attempt to disinfect seeds before germination and detect sprout contamination during production. In March 2001, we detected an increased number of *Salmonella* serotype Kottbus isolates in California. Overall, we identified 31 cases from three western states. To identify the cause, we conducted a case-control study with the first 10 identified case-patients matched to 20 controls by age, sex, and residential area. Our case-control study found illness to be statistically associated with alfalfa sprout consumption. The traceback investigation implicated a single sprouter, where environmental studies yielded *Salmonella* Kottbus from ungerminated seeds and floor drains within the production facility. Pulsed-field gel electrophoresis patterns of all patient, seed, and floor drain *Salmonella* Kottbus isolates were indistinguishable. Most implicated sprouts were from seeds that underwent heat treatment and soaking with a 2,000-ppm sodium hypochlorite solution rather than the Food and Drug Administration (FDA)-recommended 20,000-ppm calcium hypochlorite soak. Other implicated seeds had been soaked in a calcium hypochlorite solution that, when tested, measured only 11,000 ppm. The outbreak might have been averted when screening tests of sprout irrigation water detected *Salmonella* in January; however, confirmatory testing of these samples was negative (but testing improperly utilized refrigerated irrigation water). Producers should use the enrichment broth of positive screening samples, not refrigerated irrigation water, for confirmatory testing. Until other effective disinfection technologies are developed, producers should adhere to FDA recommendations for sprout seed disinfection.

On 12 March 2001, we noted an increase in *Salmonella* serotype Kottbus infections in California and identified 10 case-patients, 8 of them from southern California, all with onsets in February. Because this is an infrequent serotype of *Salmonella* in California (usually less than 10 cases are identified each year), we began an investigation to identify a possible cause of this increased number of cases.

MATERIALS AND METHODS

Case identification. California cases were identified through laboratory-based surveillance. By state regulation, all clinical *Salmonella* isolates must be referred to the California Department of Health Services Microbial Diseases Laboratory for serotyping. We also contacted health departments of other western states (Arizona, Colorado, Hawaii, New Mexico, Nevada, Oregon, Utah, and Washington) to learn if they had identified *Salmonella* Kottbus isolates after January 2001. When isolates were identified, we asked for permission to interview case-patients and for isolates to be sent to the Microbial Diseases Laboratory. All isolates were compared by pulsed-field gel electrophoresis (PFGE). We defined a case-patient as any person with a clinical specimen collected after January 2001 that yielded *Salmonella* Kottbus with the outbreak-associated PFGE pattern.

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Descriptive epidemiology. We collected demographic and clinical data for each case-patient from case-history forms submitted by local health departments. To generate hypotheses about possible exposures, we conducted exploratory interviews with five of the case-patients using an extensive, standardized survey to identify exposures to foods, restaurants, grocery stores, and travel in the week before the onset of illness.

Case-control study. To identify possible risk factors for Salmonella Kottbus infection, we conducted a case-control study. We created a standardized survey that included all food exposures reported by at least three of the five case-patients interviewed with the exploratory questionnaire. We administered this survey to case-patients and asked them about food exposures in the week before the onset of illness. Controls were selected by random-digit dialing and were matched to case-patients by sex, age group (aged 20 to 44 years or 45 to 75 years), and telephone area code and prefix. We enrolled two controls per case. Potential controls were excluded if they had a history of diarrheal illness in the month of February. We asked controls about food exposures during the period of exposure for cases (24 January 2001 to 18 February 2001).

We entered and analyzed data with Epi-Info 2000, version 1.0.5 (Centers for Disease Control and Prevention, Atlanta, Ga.). For all food exposures, we conducted matched analyses to calculate Mantel-Haenszel Matched Odds Ratios, 95% confidence intervals, and Fisher’s exact P-values. P-values ≤ 0.05 were considered significant.

Traceback investigation. We recontacted all case-patients in the case-control study who reported eating alfalfa sprouts to determine the date and location of their sprout purchase or consumption (point of service). For the six case-patients with specific recall of this information, we conducted a traceback investigation to determine the sprout grower (or growers) for each point of service. When the case-patients did not remember the date of purchase, the purchase was considered to have occurred within 1 week (for restaurant exposure) or 2 weeks (for supermarket purchase) of the date of the onset of illness. We contacted wholesalers and distributors for invoices documenting their suppliers for the period of interest. After completing the case-control study, we conducted additional tracebacks on four newly identified case-patients. When tracebacks successfully identified a common source of the alfalfa sprouts, an environmental investigation was begun.

Environmental investigation. On 29 and 30 March, we visited the production facility of the implicated sprout grower. We observed and reviewed all disinfection and sprout production practices with company officials. Seed logs, distribution records, employee time cards and work schedules, and laboratory test results of sprout irrigation water were reviewed. We assessed the available chlorine in processing water and seed disinfection solutions with a chlorine test kit (Model CN 66, Hach Co., Loveland, Colo.). We also tested for coliform bacteria in samples of well water used in the production process. We obtained environmental swabs from various areas within the production facility, including floor drains, a swamp cooler, and growing drums. These swabs were transported to the California Department of Health Services Food and Drug Partnership Laboratory (Los Angeles, Calif.) for analysis. We sampled seeds for study from unopened bags of the seedlot used during the period in which the outbreak-associated sprouts were grown. We also collected information from employees regarding diarrheal illnesses that may have occurred since 1 January 2001.

Laboratory investigation. Environmental swabs were analyzed for Salmonella by standard culture methods (1). Serogroup-
Traceback investigation. Of the seven case-patients in the California case-control study who recalled eating sprouts, five had specific recall of the place of sprout consumption or purchase. The one case-patient from Arizona also gave information regarding his sprout purchase. Traceback investigations of these six case-patients’ point of service showed one sprout grower (sprouter A) in common to five of the six persons. Subsequent traceback investigations of four additional case-patients reported later showed that all (four of four) could have eaten sprouts from sprouter A (Fig. 2).

Environmental investigation of sprouter A. Our review of sprouter A’s production records for the period during which sprouts consumed by case-patients were grown revealed the use of only one seedlot. This seedlot was imported from Australia in November 2000. The ultimate source of seed contamination was not determined. A review of sprouter A’s distribution records, as well as the purchasing histories of case-patients involved in the traceback, revealed that many of the implicated sprouts were sold by sprouter A in “clamshell” containers. According to the firm’s management, seeds for sprouts destined for these containers underwent a hybrid heat treatment/chlorination process. This combination treatment consisted of a proprietary heating process and then a cooling off in well water, followed by a 2,000-ppm sodium hypochlorite solution soak for 15 min. This hypochlorite concentration was verified during our inspection. Other sprout seeds, destined for bulk packaging in trays or bags, were treated with the FDA-recommended 20,000-ppm calcium hypochlorite solution soak for 15 min. However, the testing of this solution during our inspection showed the free hypochlorite concentration to be only 11,000 ppm.

After chlorine soaks from either of the above disinfection processes, the seeds were rinsed with well water. Because the facility is surrounded by dairy farms, we considered the possibility that their well water was contaminated by farm runoff. To explore this possibility, local county environmental health officials inspected the well heads and reviewed well-construction records. They also sampled the water supply at eight different points in the sprout producer’s distribution system. They found the well heads to be intact and in good condition. A review of the construction records indicated that the well was approximately 400 ft (122 m) deep with a sanitary seal to a depth of 130 ft (about 40 m). We also assessed the adequacy of the facility’s well water chlorination. We found a chlorine injector in the line between the well storage tank and the distribution system, but it was unclear how closely this chlorine treatment device was monitored. At the time of our inspection, the testing of well water from the distribution system within the production facility showed no residual chlorination.
A review of the firm’s sprout irrigation water screening test records revealed six *Salmonella*-positive test results using the Visual Immunoprecipitate Assay (BioControl Systems, Inc., South San Francisco, Calif.) test kits early in the period during which the outbreak-associated sprouts were grown. The first positive screening test occurred on 28 January and was followed by five more positives in the next 3 weeks. Notes made on monitoring logs indicated that all drums yielding positive screening tests contained seeds that had been treated by one of the firm’s seed disinfection procedures. To follow up each of these positive screening tests, the producer sent the retained refrigerated irrigation water sample to a private laboratory, where it was tested by FDA *Bacteriological Analytical Manual* procedures. The laboratory reported each of these retained samples as negative, and the sprouts were subsequently shipped by the producer. However, according to the test kit instructions, the correct procedure is to retain and study the enrichment broth, not the irrigation water, for confirmation of presumptive positive results.

Only two employees had unscheduled days of missed work since 6 January. Neither of these employees, nor others interviewed, reported diarrheal illness since 1 January 2001.

**Laboratory results.** Cultures of ungerminated seeds from the implicated seedlot grew *Salmonella* Kottbus. Cultures from two floor drains also yielded *Salmonella* Kottbus. One drain was located underneath the irrigation/germination drums, and the other was in the greenhouse underneather growing sprouts. Cultures of sprouts grown from this seedlot and stored at the facility were negative. Seven of the eight well water samples contained <1.1 coliform MPN/100 ml. One sample contained 2.2 coliform MPN/100 ml but <1.1 *E. coli* MPN/100 ml. Samples were not tested for *Salmonella*.

PFGE patterns of *Salmonella* Kottbus isolates from all 31 patients, floor drains, and the implicated seedlot were indistinguishable. Five background strains from other unrelated sources (included for comparison) displayed four different PFGE patterns, none of which matched the outbreak strain pattern.

**DISCUSSION**

We implicated alfalfa sprouts as the vehicle for this outbreak of *Salmonella* Kottbus infections. We identified a total of 31 case-patients, the majority of whom recalled eating alfalfa sprouts during the week before their illness. We traced these sprouts to a single grower from California. An investigation of the production facility identified the same *Salmonella* Kottbus organism in floor drains and in the ungerminated seeds of the implicated seedlot. The seeds may have been contaminated during growth, harvest, cleaning, storage, or transportation. A review of the sprouter’s production suggested that most, if not all, of these seeds had been treated with a method other than the FDA-recommended 20,000-ppm calcium hypochlorite soak and that the microbial testing of sprout irrigation water, had it been performed correctly, could have alerted the company to the presence of contamination.

*Salmonella* Kottbus infection is rare in the United States. From 1968 to 1998, an average of only 42 *Salmonella* Kottbus isolates were recovered throughout the nation each year (15). The last reported outbreak of disease caused by this organism was in 1977 in a maternity ward, where mammary shedding by one milk-donating mother had contaminated a pooled breast milk supply (10). This alfalfa sprout outbreak adds to a list of recent outbreaks caused by raw sprout consumption (8) and highlights the difficulty of ensuring the safety of this product.

Currently, there is no known method of decontamination that reliably eliminates all pathogens that can be present on sprout seeds. Disinfection of sprout seeds with 20,000 ppm calcium hypochlorite for 15 min, the currently recommended treatment, is the most effective treatment known (14). Other alternative treatments for sprout seeds, including heat pasteurization, are being studied. Jaquette et al. (6) examined the effectiveness of heat pasteurization by dipping seeds inoculated with *Salmonella* serotype Stanley in water at temperatures ranging from 54 to 71°C for 5 and 10 min. Treatment at 57 or 60°C for 5 min reduced viable counts from 263 to <1 CFU/g without reducing germination rates. Our review of available distribution records in this outbreak indicates that some, if not most, of the implicated sprouts were from seeds that had undergone the hybrid heat treatment/chlorine soak (2,000 ppm) process instead of the FDA-recommended 20,000-ppm calcium hypochlorite soak. Our findings suggest that this technique, with the time-temperature conditions used by this sprouter, did not fully inactivate pathogenic *Salmonella* from alfalfa seeds. In addition, the firm’s procedure for soaking seeds in the 20,000-ppm calcium hypochlorite solution was not adequately monitored, and when actually tested, their solution measured only 11,000 ppm.

Of the numerous sprout-related outbreaks that have occurred in the United States, only two (2, 9) reportedly involved seeds that apparently underwent a 20,000-ppm chlorine soak. However, in general, investigators of such outbreaks often have difficulty verifying the seed disinfection procedures used for the implicated sprouts, usually because of inadequate record keeping or conflicting reports of procedures by employees of implicated firms. Although other treatments of seeds or finished products such as chemical treatment, heat pasteurization, irradiation, or ozonation may prove beneficial (6, 12, 16), further study of these technologies is needed before sprout producers use them in place of FDA-recommended methodologies.

The microbial testing of sprout irrigation water has been validated as a reliable indicator of the microbiological condition of growing sprouts (4, 11) and, theoretically, can help avert sprout-associated illness. We suspect that this outbreak would have been averted, or at least mitigated, had the firm properly conducted these tests. Although the firm obtained positive Visual Immunoprecipitate Assay screening test results as early as 28 January 2001, confirmatory testing by an outside lab using FDA *Bacteriological Analytical Manual* procedures was negative. However, for
confirmatory testing, the firm did not forward the refrigerated enrichment broth from which the positive screening test was obtained. Instead, the firm forwarded the retained irrigation water sample, where, we suspect, Salmonella viability would have been compromised during the time (probably 48 h) that the irrigation water sample was held under refrigeration before shipping.

Despite press releases about these outbreaks and advisories to the public about the risk of eating raw sprouts, persons at high risk for systemic infection continue to eat sprouts. Two of the reported case-patients in this outbreak had immunocompromising conditions, and one case-patient was a young child. Both immunocompromised persons perceived raw sprouts as a “healthy” food item. Clearly, public education efforts regarding the risks of eating raw sprouts need to be continued, particularly among vulnerable populations (i.e., elderly persons, young children, and immunocompromised individuals). An example of such efforts came after this outbreak when the California Department of Health Services and the California Department of Education sent an advisory letter to all California school district superintendents and school food program managers urging them to stop serving raw sprouts to young children.

In summary, we documented a multistate Salmonella outbreak caused by contaminated alfalfa sprout seeds that were inadequately disinfected before germination. After our investigation, the sprout producer agreed to suspend the use of their hybrid heat treatment/chlorination process and to follow recommended procedures for accurately preparing and using a 20,000-ppm calcium hypochlorite soaking solution for sprout seed disinfection. The firm also agreed to follow our recommendation to amend their laboratory procedures for confirmatory testing of positive screening tests of irrigation water. All sprout producers should utilize proper procedures for such testing. The disinfection of sprout seeds should adhere to FDA recommendations, and alternative technologies should not be used until they are shown to be at least as effective as the 20,000-ppm calcium hypochlorite soak.

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