Multinational Outbreak of *Salmonella enterica* Serotype Newport Infections Due to Contaminated Alfalfa Sprouts

Chris A. Van Beneden, MD, MPH
William E. Keene, PhD, MPH
Robert A. Strang, MD, MHSc, FRCPCH
Denise H. Werker, MD, MHSc, FRCPCH
Arlene S. King, MD, MHSc, FRCPCH
Barbara Mahon, MD, MPH
Katrina Hedberg, MD, MPH
Alison Bell, MD, MHSc, FRCPCH
Michael T. Kelly, MD, PhD, FRCPCH
Vijay K. Balan, MS
William R. MacKenzie, MD
David Fleming, MD

**Context** In December 1995, reported *Salmonella enterica* serotype Newport (SN) infections increased sharply in Oregon and British Columbia but not elsewhere in North America. Similar unexplained increases had been noted in 6 other states in the fall of 1995.

**Objective** To determine the source of the outbreak(s).

**Design** Case-control studies, environmental investigations, bacterial subtyping, and surveillance information review.

**Settings** Oregon and British Columbia communities (winter 1995-1996) and Georgia, Oklahoma, Pennsylvania, Vermont, Virginia, and West Virginia (fall 1995).

**Participants** Oregon and British Columbia residents with culture-confirmed SN infections and onset from December 1, 1995, through February 29, 1996, and healthy community controls.

**Main Outcome Measures** Odds ratio (OR) of illness associated with exposures; distribution patterns and culture of alfalfa seeds and sprouts; subtyping of SN isolates.

**Results** We identified 133 cases in Oregon and British Columbia; 124 (93%) occurred in patients older than 18 years; 87 (65%) were female. Case patients were more likely than community control subjects to report having eaten alfalfa sprouts in the 5 days preceding illness (41% [17/41] vs 4% [3/75]; OR, 17.0; 95% confidence interval, 4.3-96.0). Case isolates shared a distinctive pulsed-field gel electrophoresis (PFGE) pattern. The SN was grown from seeds and alfalfa sprouts. The distribution of 1 seed lot to multiple growers corresponded to the distribution of cases. Distribution of a second seed lot from the same European wholesaler corresponded to the location of the fall outbreak, which was characterized by a similar demographic profile. The PFGE pattern of fall outbreak isolates and confiscated sprouts and seeds was indistinguishable from the Oregon and British Columbia outbreak and differed from background isolates.

**Conclusions** The SN-contaminated alfalfa seeds were distributed to multiple growers across North America in 1995 and resulted in a protracted international outbreak scattered over many months. Current sprouting methods are inadequate to protect consumers from such events. Alfalfa sprouts may be an elusive but important vehicle for salmonellosis and other enteric infections.

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States in the fall of 1995. Isolate subtyping and epidemiologic evidence suggest that both North American outbreaks were actually manifestations of a protracted outbreak caused by contaminated alfalfa seeds originally noted in Denmark in June 1995.6 These outbreaks heightened concern about the safety of a familiar food item and illustrate how contamination of a single, widely distributed product can lead to extensive, prolonged, and often difficult-to-recognize international outbreaks.

METHODS

Oregon and British Columbia Outbreaks

Salmonellosis is reportable by law to public health agencies in Oregon and British Columbia. All Salmonella isolates are serotyped at central public health laboratories. In early January 1996, laboratorians in both areas noted an increase in isolations of SN.

Case Finding and Definitions. We defined an outbreak case patient as a resident of Oregon or British Columbia with onset of diarrhea between December 1, 1995, and February 29, 1996, from whom SN was cultured.

Case-Control Studies. We conducted hypothesis-generating telephone interviews with case patients who had illness onset in December 1995. For the case-control study, we compared case patients with onset between January 1 and February 8, 1996, with 2 independent sets of control subjects. We excluded potential controls who reported having diarrhea within the preceding 30 days and those who were younger than 18 years, given the known age distribution of cases (only 7% of case patients were <18 years old). With use of a standard questionnaire, case patients and control subjects were asked about demographic characteristics and—for the 5 days before illness onset (cases) or interview (controls)—meals and snacks eaten away from home, travel, and consumption of an extensive list of specific food items.

Salmonellosis Controls. We surveyed currently healthy adults who had been previously diagnosed as having salmonellosis in 1995 as identified from provincial and state laboratories databases.

We excluded those whose infections had been caused by serotype Newport or Stanley. (An outbreak of serotype Stanley infections elsewhere had been linked to sprout consumption in 1995.)

Community Controls. We obtained a second set of controls by interviewing adults reached at randomly selected telephone numbers from the Portland, Ore, and Vancouver, British Columbia, residential directories.

Environmental Investigations and Tracebacks. We traced the distribution of sprouts eaten by case patients from retailers and restaurants back to local sprout growers and finally to alfalfa seed distributors. Public health inspectors reviewed food-handling practices at restaurants and sprouting facilities, collecting samples of seeds and sprouts when possible. We also interviewed staff at each restaurant identified by a case patient to determine if alfalfa sprouts were served on any menu items.

Multistate Outbreak, Fall 1995

Background. At the time of the Oregon–British Columbia outbreak, federal epidemiologists at the CDC and at the Laboratory Centre for Disease Control in Ottawa, Ontario, were unaware of any concurrent unusual SN activity. Six states, however, had reported marked increases in SN cases between August and October of 1995 (Laurence Slutsker, MD, CDC, oral communication, January 1996). A case-control study among Vermont college students with diarrhea, including 2 with SN infections, had implicated alfalfa sprouts as the probable source (Susan Schoenfeld, RN, MSPH, Vermont Department of Health, oral communication, January 1996). In the other 5 states, no specific sources had been identified, and the outbreaks had not been linked to each other. We hypothesized that contaminated alfalfa sprouts had caused these earlier outbreaks.

Case Finding: Case and Control Definitions. In the United States, state public health laboratories report all Salmonella isolates and serotypes to CDC through the Public Health Laboratory Information System. We reviewed summary data for 1995 SN infections. Information was available for 43 of 50 states. We defined outbreak states as those with a greater than 10-fold increase in reported SN incidence during the fall outbreak period (August–October 1995) relative to the rates from January through July 1995. We defined control states as those reporting a less than 4-fold increase, excluding states with 4- to 9-fold increases.

Case-Control Study. In 1995, more than 70% of alfalfa seed sold to sprout growers in the United States came from a single distributor. We reviewed shipping records from this distributor and compiled a list of seed lots shipped to sprout growers throughout the United States during the outbreak period. In a case-control analysis, we compared the distribution of specific seed lots with outbreak and control states, excluding states receiving no seeds from the national distributor during this period.

Laboratory Investigations. In Oregon, alfalfa sprouts and seeds were screened for Salmonella with use of a commercial enzyme-linked immunosorbent assay kit following broth enrichment, followed by standard culture methods.8,9 Canada’s Health Protection Branch used preenrichment followed by selective enrichment and streaking onto selective agars.10 Confiscated seeds were also germinated in the laboratory and subsequently cultured.9,10 The SN isolates were characterized by pulsed-field gel electrophoresis (PFGE), cutting with XbaI,11 and bacteriophage typing12 as previously described.

Statistical Analysis

Data were analyzed using the χ2 test, the 2-tailed Fisher exact test, or stratified analysis as appropriate.13

RESULTS

Oregon and British Columbia Outbreak

Descriptive Epidemiology. We identified a total of 133 case patients, ranging in age from 1 day to 94 years (median, 36 years); 124 (93%) were aged 18 years or older. Eighty-seven (65%) were female. (In contrast, in 1995 only 62% of the 1213 reported salmonellosis cases in Oregon and British Columbia were female.)

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CONTAMINATED ALFALFA SPROUTS

aged ≥18 years; 50% were female.) All patients reported diarrhea. Ten percent of the outbreak case patients were hospitalized; there were no deaths.

Geographically, cases were scattered across western Oregon and southern British Columbia. Most case patients reported onsets of symptoms in December and January (Figure). Almost no case patients were linked by exposure to shared meals at restaurants, other public gatherings, or within a household.

**Case-Control Studies.** Although case patients were not more likely than either set of control subjects to have eaten at a particular restaurant, they were more likely to have eaten meals away from home in general (95% of case patients vs 74% of salmonellosis controls [odds ratio (OR), 6.7; 95% confidence interval (CI), 1.5-61.0]) and 57% of community controls [OR, 14.5; 95% CI, 3.3-131.0]). Alfalfa sprouts were the only item from among a list of more than 70 foods and drinks that was statistically associated with illness compared with both foods and drinks that was statistically associated with illness compared with both sets of controls, although only a minority of case patients in the case-control study recalled sprout consumption in the 5 days before onset (41% of 41 case patients reported eating sprouts vs 10 (12%) of 86 salmonellosis controls (OR, 5.4; 95% CI, 2.0-15.0) and 3 (4%) of 75 community controls (OR, 17.0; 95% CI, 4.3-96.0). (Another 4 cases reported eating foods that, according to restaurant staff, always included alfalfa sprouts.) At least 26 case patients (63%) ate at a restaurant that served alfalfa sprouts, compared with 17 (20%) of 86 salmonellosis controls. Similar data were unavailable for community controls.

The incubation periods for the 16 case patients for whom a unique sprout consumption date could be determined ranged from 12 hours to 5 days (mean, 3.5 days; median, 4 days).

**Environmental Investigations and Tracebacks.** Alfalfa sprouts consumed by 8 of 11 Oregon case patients with a single identifiable source were traced back to 1 Portland grower. During the outbreak period, lot A was identified as the only source of seed for this grower. Approximately 90% (16 340 kg) of lot A was shipped from the distributor to growers in Oregon and British Columbia. The restriction of cases to Oregon and British Columbia was thus explained by the distribution and sales of alfalfa sprouts germinated from a single seed lot, lot A. No noteworthy irregularities were found on inspection of this or any other sprouting facility involved in this outbreak.

The national distributor purchased alfalfa seed from multiple sources in several countries. Lot A seed came from another distributor in the Netherlands. No obvious evidence of contamination or mishandling was found at either distributor’s facility. It was impossible to identify the farm even the continent from which lot A seed originated; adequate records were not available from the Dutch facility.

**Control Measures.** Through media releases on February 8, 1996, we notified the public about the outbreak and its association with alfalfa sprouts. Health officials elsewhere in Canada and the United States were also alerted. Unsold alfalfa sprouts and approximately 9100 kg of unsprouted lot A seed were recalled. Reported SN infections soon declined to preepidemic levels (Figure).

**Laboratory Investigations.** Culture of Alfalfa Seed and Sprouts. The SN was grown from 1 of 52 samples of sprouts (an opened package) and none of 31 samples of lot A seed taken from grocery stores, homes, restaurants, and sprout growers in Oregon and British Columbia. *Salmonella* Newport was also grown from unsprouted lot A seed confiscated from a grower in California (Gregory Inami, BA, Microbial Diseases Laboratory, California Department of Health Services, oral communication, June 1997).

**Subtyping.** We subtyped 80 SN isolates from Oregon and British Columbia by PFGE. Thirty-five (81%) of 43 isolates obtained during the outbreak period were indistinguishable (the “outbreak pattern”); 3 other isolates differed by a single band. In contrast, the outbreak pattern was not seen in any of 19 preoutbreak isolates tested and in only 4 of 18 postoutbreak isolates. *Salmonella* Newport isolates cultured from confiscated sprouts and lot A seed shared the outbreak pattern. Nonoutbreak pattern isolates were extremely heterogenous.

We phage typed 96 SN isolates from Oregon and British Columbia; 83 (93%) of 89 outbreak period isolates were type 2, compared with only 1 of 7 preoutbreak isolates (that one coming just 1 week earlier). All isolates with the outbreak PFGE pattern or its close variant tested were phage type 2. All other isolates were phage type 4, 5, 8, or 14 or were untypeable.

**Multistate Outbreak, Fall 1995**

**Case-Control Study.** We identified 6 outbreak states (Vermont, Pennsylvania, Georgia, Oklahoma, Virginia, and West Virginia) and 13 control states. The same national distributor shipped alfalfa seeds from 29 uniquely numbered lots to growers in these 19 states during August and September 1995. The mean number of sprout growers in outbreak

**Figure.** Epidemic Curve by Week of Onset, January 1995-June 1996

The curve shows confirmed cases of *Salmonella enterica* serotype Newport infection in Oregon and British Columbia (BC). Cases increased sharply in early December 1995 and then declined dramatically after recall of alfalfa seeds and sprouts.
and control states was similar (2.3 and 2.5, respectively), as was the mean quantity of seed shipped to growers in those states (3235 kg and 2433 kg, respectively). The distribution of only 1 lot, lot Z, was strongly associated with outbreak occurrence. Lot Z was shipped to 5 (83%) of 6 outbreak states and 1 (8%) of 13 control states (OR, 60; 95% CI, 2.2-3056.0). (Most of the sprouts sold in West Virginia—the 1 state that did not get lot Z seed—came from a grower in neighboring Pennsylvania that did receive seed from lot Z.) Lot Z was purchased by the same national distributor from the same Dutch seed broker as lot A, and was similarly untraceable to the continent or farm of origin.

**Laboratory Investigations.** Subtyping. By PFGE, we subtyped 31 SN isolates obtained from residents of the fall outbreak states (provided by public health laboratorians of those states and CDC). None of 6 isolates that were collected before distribution of lot Z seed matched the outbreak pattern, whereas 22 (85%) of 25 isolates obtained during the fall outbreak period were indistinguishable from the outbreak pattern. No lot Z seeds were available for microbiological testing. A representative isolate from the June 1995 Danish outbreak also matched the Oregon–British Columbia outbreak pattern.

**COMMENT**

Consumption of a single lot of contaminated alfalfa seeds caused a large outbreak of salmonellosis in Oregon and British Columbia. Illness was strongly associated with sprout consumption, SN with the outbreak PFGE pattern was isolated from almost all outbreak-associated cases and from left- over sprouts and seeds, and SN infections rapidly declined following recall of the implicated lot. Temporal and geographical clustering of cases in 2 jurisdictions clearly indicates that the source of contamination was the seed itself and not contamination that occurred during production or mishandling of the final product.

Further evidence indicates that contaminated seed from the same distributor caused the previously unexplained SN outbreaks in other states during the fall of 1995. First, distribution of a different seed lot was statistically associated with the occurrence of large SN outbreaks with a similar demographic profile. Second, the PFGE patterns of isolates from these outbreaks matched the Oregon–British Columbia pattern, whereas the PFGE patterns from SN isolates collected before lot Z was distributed were different.

The knowledge that early in 1995 Denmark had implicated alfalfa sprouts in an SN outbreak greatly facilitated the focus of our investigation. The demographic profile and PFGE fingerprints of the Danish outbreak matched those found in Oregon and British Columbia.

Collectively, these observations indicate that alfalfa seeds, most likely contaminated from a single source, were distributed through multiple channels first to growers in Europe and then to North America, causing numerous localized manifestations of 1 large outbreak that extended over at least 9 months. Seeds grown on different farms are commonly mixed. Lot numbers only correspond to shipping units, not processing or harvesting units; seeds from the same source could be shipped as multiple lots.

Several epidemiologic features of this investigation are worth highlighting. First, although finding a preponderance of adults (and particularly women) is neither a necessary nor sufficient criterion of a sprout-associated outbreak, it has been noted repeatedly; presumably women have different eating habits than men. Second, the median incubation period for these cases exceeded the maximum cited in a standard communicable disease reference. Had we restricted food histories to eating these contaminated alfalfa sprouts in North America alone, as fewer than 5% of Salmonella infections are thought to be reported. Since the SN epidemic, outbreaks of salmonellosis caused by different serotypes have been traced to alfalfa sprouts at least 4 times in North America (W.E.K., unpublished data, October 1997). In almost every instance, investigations were triggered because of an increase in reports of relatively uncommon serotypes. This not only underscores the value of routine serotyping but also suggests that similar outbreaks caused by more common serotypes would be easy to miss. Alfalfa sprouts have also been implicated in a recent outbreak of Escherichia coli O157:H7 infections.

Alfalfa sprouts are a well-suited vehicle for salmonellosis. Seeds are often stored for months or years under cool, dry conditions in which salmonellae are stable. During the 3- to 5-day sprouting process, numbers of salmonellae may increase by 3 to 4 orders of magnitude, decreasing little if at all during subsequent refrigeration. Alfa sprouts are rarely washed or cooked before consumption, and consumers are left with little protection other than chance.

From farm to table, many opportunities exist for contamination of alfalfa seeds or sprouts. Crops can be easily contaminated with dirty water, runoff from adjacent farms, animal fertilizers used in previous growing seasons, or droppings from

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rodents or ruminants. Assurance of a clean seed would require intensive monitoring of farmlands and dedication of fields and harvest machinery to a crop of seeds destined for sprouting. As only a minority of alfalfa seed production is earmarked for sprouting and therefore human consumption, the above methods might prove too expensive for farmers. Furthermore, few regulations are currently consistently applied to all sprout growers. In fact, whether a grower is considered a food producer or a farmer varies from state to state. Some sprout distributors and growers are currently testing seed samples prior to distribution or sprouting. As contamination may be intermittent and low level, this testing is probably ineffective. For example, lot A seed samples cultured by the national distributor tested negative for Salmonella. Although “downstream” preventive efforts have been studied more closely, the fundamental problem is that the spraying process contains no “kill step” that would eliminate pathogens without compromising a seed’s germination potential. Calcium hypochlorite, sodium hypochlorite, hydrogen peroxide, and ethanol have all been tested as methods of seed decontamination before spraying. These treatments can significantly reduce populations of Salmonella inoculated onto alfalfa seeds without adversely affecting germination. However, viable salmonellae can still be detected after treatment, indicating the potential for multiplication during sprouting. Salmonella organisms likely reside in seed crevices and between the cotyledon and testa, areas not reached by chemical treatments. Irradiation is currently being evaluated as an adjunct seed decontamination method. Until barriers to a pathogen-free seed are resolved, however, we conclude that alfalfa sprouts are a high-risk food for salmonellosis. All consumers, particularly those at greatest risk for severe disease (immunocompromised, elderly, and very young people), should consider this danger when deciding whether to eat alfalfa sprouts.

Author Affiliations: Oregon Health Division, Portland (Drs Van Beneden, Keene, Hedberg, and Flenn); Epidemiologic Service, Epidemiology Program Office, Centers for Disease Control and Prevention, Atlanta, Ga (Drs Van Beneden); Department of Health Care and Education, University of British Columbia, Vancouver (Drs Strang, King, and Bell); British Columbia Centre for Disease Control, Epidemiology Services, Vancouver (Drs Werker, King, and Bell); Field Epidemiology Training Program, Laboratory Centre for Disease Control, Health Canada, Ottawa, Ontario (Dr Werker); Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control, Washington, DC; Debbie Emery, MD, County Health Department, Louisville, Ky; Oregon Department of Agriculture, Salem; Gregory Inam, BA, Microbial Diseases Laboratory, California Department of Health Services; Susan Schoenhofer, PhD, Montana Department of Health; Paul Blake, MD, Division of Public Health, Department of Human Resources, Atlanta; Catherine Slep, MD, and Carl Bernyam, DVM, West Virginia Department of Health and Human Resources, Charleston, Marshal Deasy, Pennsylvania Department of Health, Harrisburg; Henrik Wegener, PhD, Danish Veterinary Laboratory, Copenhagen; Arjali Deshpande, MPH, Oregon State Department of Health, Oklahoma City; and the medical health officers and staff from the numerous health units and departments in British Columbia and Oregon.

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