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Temporally distinct *Escherichia coli* O157 outbreaks associated with alfalfa sprouts linked to a common seed source – Colorado and Minnesota, 2003

D. D. FERGUSON[1]*, J. SCHEFTEL[2], A. CRONQUIST[3], K. SMITH[2], A. WOO-MING[3], E. ANDERSON[2], J. KNUTSEN[3], A. K. DE[4] AND K. GERSHMAN[3]

[1] *Epidemic Intelligence Service assigned to the Colorado Department of Public Health and Environment, Denver, CO; and Office of Work Force and Career Development, Centers for Disease Control and Prevention, Atlanta, GA, USA*
[2] *Minnesota Department of Health, Minneapolis, MN, USA*
[3] *Colorado Department of Public Health and Environment, Denver, CO, USA*
[4] *Office of Work Force and Career Development, Centers for Disease Control and Prevention, Atlanta, GA, USA*

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SUMMARY

Escherichia coli O157 outbreaks were identified in Minnesota in February 2003 involving seven persons and in Colorado in July 2003 involving 13 persons. Case isolates from the two states had matching pulsed-field gel electrophoresis (PFGE) patterns. Independent case-control studies linked infections in each outbreak with eating alfalfa sprouts that were traced to the same seed distributor. The Colorado sprouter reportedly complied with the Food and Drug Administration (FDA) sprout guidance, whereas the Minnesota sprouter did not. These investigations revealed that increased compliance with existing FDA guidance is needed and that additional research is needed to improve the alfalfa seed decontamination process. This reaffirms the FDA recommendation that raw alfalfa sprouts should be considered potentially contaminated and avoided by persons at high-risk such as the elderly, young children, and immunocompromised persons. PFGE played an essential role in linking these two temporally and geographically distinct *E. coli* O157 outbreaks.

INTRODUCTION

Escherichia coli O157:H7 is a common cause of diarrhoea, causing an estimated 75000 illnesses a year in the United States [1]. It is the leading cause of haemolytic–uraemic syndrome (HUS) and paediatric renal failure [2]. Initial *E. coli* O157 outbreaks were associated with ground beef and unpasteurized dairy products. Recent outbreaks have also been linked to produce, including lettuce, alfalfa sprouts, unpasteurized apple cider, and apple juice [2].

* Author for correspondence: D. D. Ferguson, M.D., CDPHE, 4300 Cherry Creek Drive, South, Denver, CO 80246, USA. (Email: dayna.ferguson@state.co.us)

E. coli O157:NM is a non-motile form of *E. coli* O157 also referred to as non-motile *E. coli* O157 [3], *E. coli* O157:non-motile [4], and *E. coli* O157:H$^-$ [5]. A 1995 outbreak of *E. coli* O157:NM infections in Germany resulted in 28 HUS cases demonstrating that it can have similar pathogenicity as *E. coli* O157:H7 [6].

During 1998–2002, an average of 110 cases of *E. coli* O157 per year were documented in Colorado [7] and 196 in Minnesota [8]. In February 2003, the Minnesota Department of Health (MDH) identified a cluster of seven *E. coli* O157 isolates through routine surveillance. Although four isolates were motile and three non-motile, all were indistinguishable by

pulsed-field gel electrophoresis (PFGE) using the restriction enzyme *Xba*I. In July 2003, the Colorado Department of Public Health and Environment (CDPHE) identified a cluster of 13 *E. coli* O157:NM isolates through routine surveillance. The Colorado isolates' *Xba*I PFGE pattern was indistinguishable from Minnesota's outbreak pattern. This report summarizes these outbreak investigations, both linked to alfalfa seeds from a single seed distributor, adds to mounting evidence that contaminated alfalfa sprouts are an important source of foodborne disease, and illustrates how contaminated foods can cause geographically widespread outbreaks in a global market.

METHODS

E. coli O157 is a nationally notifiable infection in the United States [9]. Laboratories in Minnesota and Colorado report culture-confirmed cases to health departments and send isolates to state laboratories for PFGE subtyping with *Xba*I [10]. State and local health departments routinely contact identified patients to obtain demographic information, exposure histories, and symptoms.

Epidemiological methods – Minnesota investigation

On 14 February 2003 through routine laboratory surveillance, the MDH identified a cluster of five *E. coli* O157 case isolates with indistinguishable *Xba*I PFGE patterns. The MDH conducted a case-control study based on hypotheses generated from standard enteric pathogen interviews of the first four patients. A case was defined as culture-confirmed *E. coli* O157 infection with an isolate matching the outbreak *Xba*I PFGE pattern in a Minnesota resident with illness onset after 1 January 2003. Two age-matched controls were selected for each patient by sequential digit dialling anchored to a case's telephone number. Controls were asked about food and restaurant exposures for the week before the matched patient's illness, using the same questionnaire format as for cases. Interviews included questions about food consumption at 22 chain restaurants because case interviews suggested a common exposure to one of two restaurant chains. Potential controls who travelled outside Minnesota, had diarrhoea ($\geqslant$3 loose stools/24 h), or reported household contacts infected with *E. coli* O157 were excluded.

Epidemiological methods – Colorado investigation

A case was defined as an *E. coli* O157:NM infection with an isolate matching the outbreak PFGE subtype in a Colorado resident with illness onset during 18–30 July 2003. The CDPHE and local health department staff interviewed patients identified through routine laboratory surveillance, using a hypothesis-generating questionnaire to obtain demographic information, symptoms, and exposure histories for the week preceding illness. Questions included general recall of foods and restaurants and specific recall of 23 foods.

Two matched controls per case were selected based on sex and age range (18–40 and 41–65 years). Controls were interviewed using the same format as for patients about exposures during 14–20 July (the week before the outbreak peak). Controls were contacted using sequential digit dialling based on patients' phone numbers. When calls resulted in a business, busy signal, recording, or refusal, interviewers called the next number. Potential controls who had intestinal illness in July or travelled outside of Colorado for more than 1 day during 14–20 July were excluded.

Laboratory methods – Minnesota investigation

Routine PFGE subtyping after digestion with *Xba*I (Promega Corporation, Madison, WI, USA) was performed on all *E. coli* O157 isolates received at MDH [10]. Seven isolates with the *Xba*I outbreak pattern were also tested by PFGE with *Bln*I (Roche Molecular Biochemicals, Mannheim, Germany) [10]; the *Xba*I pattern was posted on PulseNet, the National Molecular Subtyping Network for Foodborne Disease Surveillance.

Laboratory methods – Colorado investigation

The CDPHE laboratory performed PFGE subtyping with *Xba*I and *Bln*I [10]. *E. coli* O157:NM isolates with matching *Xba*I PFGE patterns were identified as potential cases, and the pattern was posted on PulseNet.

Sprouts, seeds, water samples, and environmental swabs collected at the implicated sprouter facility by the Colorado Consumer Protection Division (CPD) were cultured and analysed using the EHEC Enrichment broth-Tellurite-Cefixime-Sorbitol MacConkey (EEB-TCSMAC) enrichment and isolation method (Dynal Inc., Lake Success, NY, USA) [11].

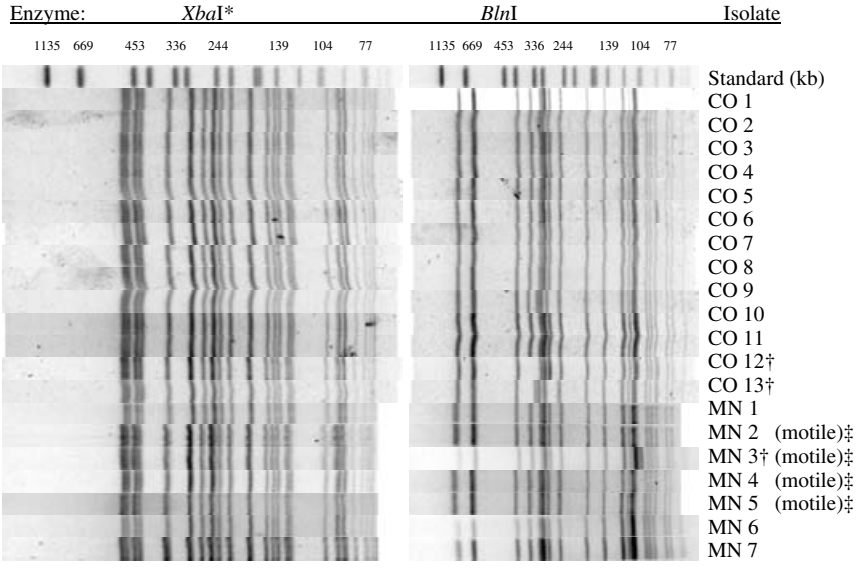

Fig. 1. Pulsed-field gel electrophoresis patterns for Colorado and Minnesota isolates of *Escherichia coli* O157. * All *Xba*I PFGE patterns are indistinguishable. † *Bln*I PFGE patterns differ from predominant *Bln*I pattern by one band. ‡ Motile isolates are identified. All other isolates were non-motile.

Trace-back methods – Minnesota investigation

Investigators from the Minnesota Department of Agriculture (MDA) and sanitarians from Minneapolis Environmental Health and Hennepin County Epidemiology and Environmental Health conducted a trace-back investigation based on purchase records and inspected the implicated restaurants, sprout distributor and sprouter. At the sprouter facility, the MDA collected two alfalfa seed samples, two sprouted seed samples, two irrigation water samples, and eight product samples from alfalfa sprout pallets in the cooler. Additionally, the Food and Drug Administration (FDA) inspected the implicated sprouter and seed distributor facilities. The FDA collected 26 environmental samples from the sprouter facility but no seed, sprout, or environmental samples from the seed distributor.

Trace-back methods – Colorado investigation

The CPD conducted a trace-back investigation based on purchase records and inspected implicated food establishments, sprout distributors, and sprouters. They randomly collected seven sprout samples, two seed samples, two spent irrigation water samples, and 11 environmental samples from the sprouter facility. Seeds and sprouts from the implicated lot were unavailable for testing. The sprouter's culture records from spent irrigation water samples were reviewed. FDA investigators inspected the implicated sprouter and seed distributor facilities. No seed, sprout, or environmental samples were collected from the seed distributor.

Statistical methods – Colorado and Minnesota

Mantel–Haenszel matched odds ratios (mOR) with exact 95% confidence intervals for maximum-likelihood estimate (CI) was used for bivariate measures of association. *P* values were determined using the Mantel–Haenszel summary χ^2 (Epi-Info 6, Centers for Disease Control and Prevention, Atlanta, GA, USA). Multivariate analysis was performed using Exact Logistic Regression (LogXact, 4.1, Saugus, MA, USA) and adjusted odds ratios (aOR) were derived from the conditional maximum-likelihood estimate.

RESULTS

Minnesota investigation

Seven culture-confirmed cases were identified in Minnesota, including five primary and two secondary cases. Four patients had motile isolates; two primary patients and one secondary patient had non-motile isolates. All seven isolates had indistinguishable *Xba*I PFGE patterns, and six out of seven had indistinguishable *Bln*I PFGE patterns. A single band difference was observed between one primary motile isolate and the outbreak *Bln*I pattern (Fig. 1). Based

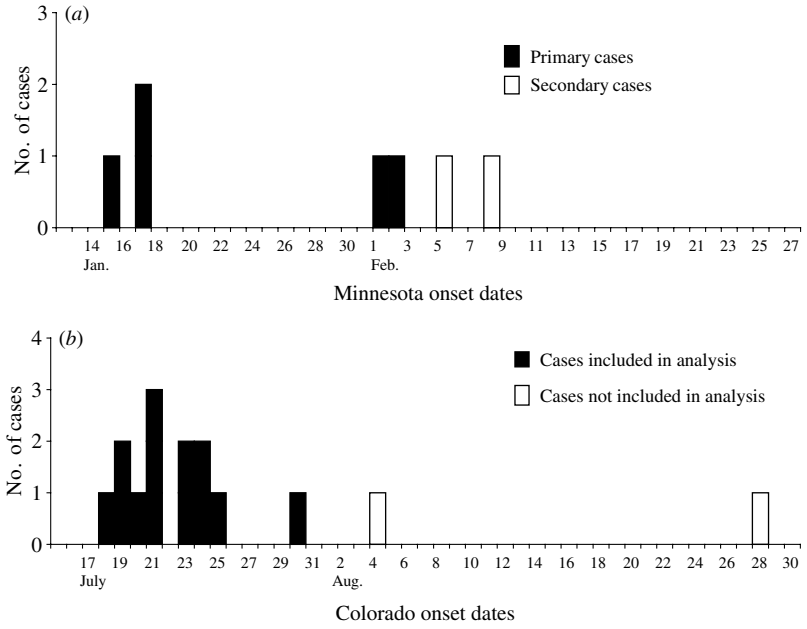

Fig. 2. Cases of *Escherichia coli* O157 infection associated with alfalfa sprout consumption by date of onset. (*a*) Minnesota and (*b*) Colorado outbreaks, 2003.

Table. *Characteristics of case patients with* Escherichia coli *O157 infections in Colorado and Minnesota outbreaks, 2003*

Characteristic	Colorado ($n=13$)	Minnesota ($n=7$)
Median age (range), years	24 (19–64)	30 (21–57)*
Female (%)	7 (54)	5 (71)
Reported signs and symptoms (%)		
Diarrhoea	92	100
Bloody diarrhoea	77	100
Abdominal cramps	100	100
Vomiting	62	14
Fever	46	57
Hospitalized	8	29
Haemolytic–uraemic syndrome	0	0

* Only includes five primary cases.

on PulseNet records, these patterns had not been identified previously in the United States.

Illness onset for primary patients ranged from 15 January to 2 February 2003 (Fig. 2); the median age was 30 years (range 21–57 years) (see Table). Two patients were hospitalized; two visited emergency departments; no deaths or HUS cases were reported.

Three primary patients reported eating alfalfa sprouts at one of three restaurants belonging to two chains. A fourth regularly ate alfalfa sprouts on sandwiches from one of the same chain restaurants but could not recall eating there the week before illness onset. A fifth patient was a male aged 6 years with illness onset on 8 February. His mother reported a diarrhoeal illness beginning 1 February after eating alfalfa sprouts at one of the same chain restaurants on 30 January. The mother was classified as the fifth primary case, and her son and daughter aged 3 years (became ill 5 February) were classified as secondary cases. *E. coli* O157 isolates from samples submitted by the daughter and mother were non-motile and by the son motile. No sprout exposure was reported for either child. All five primary cases ate at restaurants in the Minneapolis–St. Paul metropolitan area in the week before their illness.

Food histories suggested alfalfa sprouts were a common food vehicle. Three out of four primary patients reported eating them compared with 0 out of 8 controls (mOR undefined, 95% CI lower limit 1·7, $P=0·01$). No other food items were significantly associated with infection among 60 items surveyed (15 fruits and juices, 21 vegetables, 9 salads, 10 meats and 5 other foods).

Alfalfa sprouts from four identified restaurants were traced to the same food distributor, to a single sprouter in Minneapolis, and to a single seed distributor (Fig. 3). No deficiencies were identified by sanitarians at food establishments or sprout distributors.

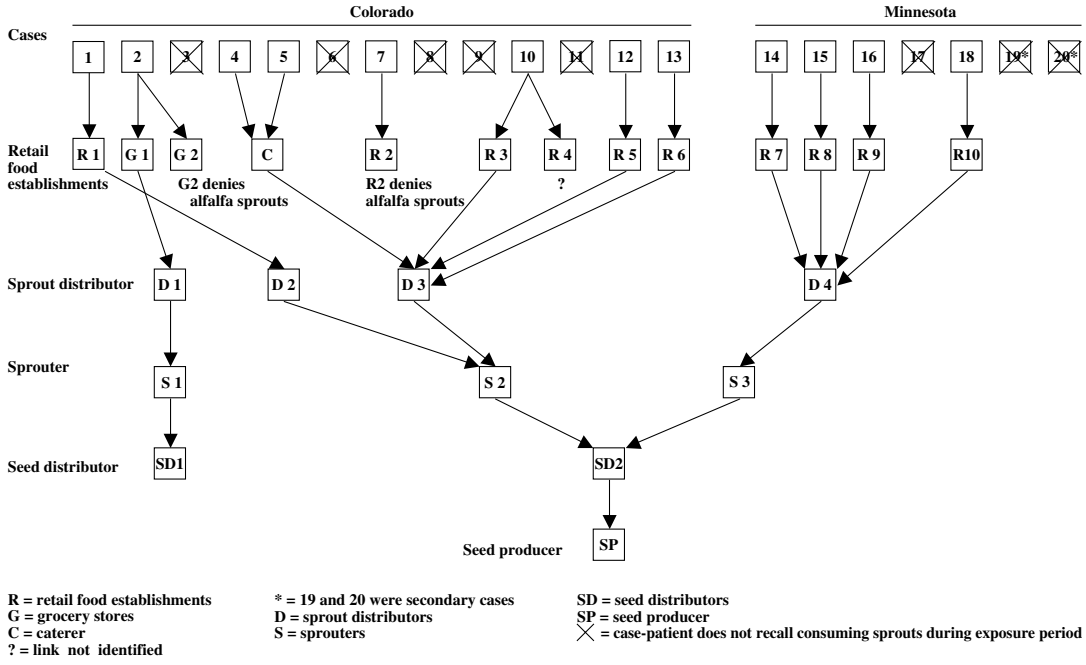

Fig. 3. Trace-back investigations of *Escherichia coli* O157 outbreaks in Colorado and Minnesota associated with alfalfa sprout consumption.

Implicated sprouts originated from 5400 kg of seeds that the sprouter received from the distributor in December 2002. The seeds were from a single 20 000 kg lot imported from Australia and distributed nationwide with only 100 kg remaining at the distributor plant. The Minneapolis sprouter subsequently returned 3600 kg of remaining seeds to the distributor in March 2003. The MDA recommended that unused seeds from other states also be returned. According to the FDA, implicated seeds were diverted to agricultural uses. The MDA's cultures of alfalfa sprouts, irrigation water, and seed samples from the sprouter facility were negative for *E. coli* O157. Results of the FDA's environmental samples could not be obtained.

During the sprouting plant inspection, the MDA identified three problems: inadequate hypochlorite concentration (15 000 ppm, not the recommended concentration of 20 000 ppm), inadequate agitation of disinfecting solution, and improper testing of spent irrigation water (weekly testing, not by production lots as recommended) [12]. The sprouter voluntarily halted alfalfa sprout production, disinfected the plant, and then resumed production with a new seed lot after re-inspection.

Colorado investigation

Thirteen Colorado residents were identified with *E. coli* O157:NM isolates with matching PFGE patterns during 18–30 July (Fig. 2). Colorado's outbreak pattern was indistinguishable from Minnesota's by *Xba*I, and 11 out of 13 isolates were indistinguishable by *Bln*I, suggesting that these infections originated from a common source. The remaining two isolates differed by a single band from the *Bln*I outbreak pattern (Fig. 1).

Two additional patients not included in this analysis were later identified with matching *E. coli* O157:NM isolates. One was a woman aged 28 years with illness onset on 4 August 2003. She was not interviewed until January 2004 and did not recall eating alfalfa sprouts. The other patient was a female aged 1 year with illness onset on 28 August 2003. Her parents reported eating alfalfa sprouts the week before her illness but were asymptomatic and unable to specify where they purchased sprouts. Based on PFGE patterns submitted to PulseNet, no other *E. coli* O157 isolates with matching patterns were identified in the United States during 2003.

Case patients' median age was 24 years (range 19–64 years); 54% were female. One person was hospitalized; no deaths or HUS cases were reported (see Table). Two patients' exposures occurred in Wyoming, two in southwestern Colorado, and the rest in the Denver metropolitan area. The two patients in Wyoming were Colorado residents working as fire fighters, whose food was purchased in Colorado by a caterer.

Routine case interviews suggested that alfalfa sprouts were a common food exposure eaten by 8 out of 13 (62%) patients. Among 23 food items (9 fruits, 8 vegetables, 6 meats), only alfalfa sprouts were significantly more likely to have been eaten by patients than controls [8/13 (62%) *vs.* 2/26 (8%); mOR 8·0, 95% CI 6·0–77·3]. Although patients were also more likely to have eaten red grapes than controls [6/13 (46%) *vs.* 4/26 (15%)], this association was not statistically significant. Using exact multiple logistic regression, alfalfa sprouts remained statistically significant (aOR, 7·4, 95% CI 1·4–74), whereas red grapes were not (aOR 7·9, 95% CI 0·62–466).

Six out of eight (75%) patients who recalled consuming alfalfa sprouts did so at one of four retail food establishments or one caterer (Fig. 3). One sprouter supplied alfalfa sprouts to these five businesses. This sprouter's seeds received in June 2003 were traced to one lot from the same seed distributor identified in the Minnesota outbreak.

Of two other patients who recalled consuming alfalfa sprouts, one identified two grocery stores where he might have obtained them. One store purchased sprouts that were traced to a seed producer in New Mexico; the second denied selling alfalfa sprouts. The other patient reported eating alfalfa sprouts at an establishment that also denied serving sprouts.

The trace-back investigation identified no deficiencies at food establishments, sprout distributors, or the sprouter facility. The sprouter reported following FDA seed decontamination guidelines, agitating seeds in calcium hypochlorite (20 000 ppm) for 15 min. No samples from the sprouter grew *E. coli* O157, but no implicated seeds were available for testing. Spent irrigation water samples tested by the sprouter prior to the outbreak were negative for *E. coli* O157.

The FDA only released a redacted copy of their Establishment Inspection Report to CDPHE and MDH, because it was considered confidential commercial information. Implicated seed lots in Minnesota and Colorado were imported from a 2002 crop grown in Australia and were at the distribution plant concurrently. Plant management assured FDA investigators that these seed lots were not inadvertently mixed. The seed distributor's records indicated that microbiological testing of 5-lb samples drawn randomly from 10% of seed bags, and sprout water samples obtained from sprouting seed from implicated lots were negative for *E. coli* O157. However, no implicated seeds were available for testing by the FDA. No specific deficiencies were identified at the seed distributor's plant. The FDA reported that because there was no memorandum of understanding between Australia and the FDA that allowed exchange of confidential commercial information, the trace-back investigation did not extend to Australia and no international trace-forward investigation was conducted.

DISCUSSION

These investigations produced strong epidemiological and laboratory evidence implicating alfalfa seeds from the same distributor as the cause of two geographically and temporally distinct outbreaks of *E. coli* O157. Matching PFGE patterns and epidemiological evidence from the Minnesota outbreak in February, and the Colorado outbreak in July, point to a common source of infection. The only common denominators in these outbreaks were the seed distributor and seed source (Australia). Thus, contamination must have occurred at or before the level of the seed distributor, rather than during sprouting, packaging, distribution, or food service. Possible scenarios include (1) implicated seeds in Minnesota and Colorado originated from the same Australian fields and moved independently through the seed distributor; (2) implicated seeds from the Minnesota outbreak were incorporated into the implicated Colorado seed lot; or (3) persistent or recurrent environmental contamination with *E. coli* O157 at the seed distributor.

There was considerable delay and difficulty in obtaining information from the FDA regarding their investigation. The reason cited by FDA officials was that current laws prohibit the FDA from releasing confidential commercial information even during outbreak investigations. When foodborne outbreaks involve multiple states, the FDA plays a crucial role in trace-back investigations. A legal mechanism is needed to facilitate timely communication of FDA findings back to state health departments.

Similarly, in a global economy, diplomatic agreements that allow information exchange between countries regarding disease outbreaks are essential to identify causes and protect the public from further outbreaks. For example, although rare in the United States, *E. coli* O157:NM is more common than *E. coli* O157:H7 in Australia [13, 14]. Determining whether contamination occurred in Australian fields, what factors might have caused contamination, and whether

other countries were involved is vital to enable implementation of effective control measures.

The percentage of patients who reported eating alfalfa sprouts in the Colorado outbreak (62%) was significantly higher than the typical percentage of Colorado residents who eat alfalfa sprouts. In a 2002 random-digit phone survey of Colorado residents, 4·9% of 911 respondents reported eating alfalfa sprouts in the week before the survey [15]. These data facilitated hypothesis generation in the Colorado investigation and might be useful for investigating other foodborne outbreaks.

The combination of motile and non-motile *E. coli* O157 isolates recovered in Minnesota is interesting. The link between a mother with a non-motile isolate and her son with a motile isolate and matching PFGE patterns suggests that these isolates were from the same outbreak despite the phenotypic difference. It is unclear whether the expression of motility resulted from a phenotypic switch or a genetic mutation.

PFGE subtyping was essential in these investigations. It increased the case definition specificity so that alfalfa sprouts were identified as the food vehicle with relatively few cases. The utility of PFGE subtyping in routine surveillance and in identifying geographically widespread outbreaks has been demonstrated previously [16–22]. In the Minnesota investigation, PFGE was also essential in linking phenotypically different isolates of *E. coli* O157.

Also noteworthy was that although geographically widespread, these outbreaks were limited to few persons. It is possible that infections were more widespread than reported. Studies from FoodNet sites suggest that for each reported *E. coli* O157 infection, 13–27 actually occur [1]. Alternatively, seed decontamination with calcium hypochlorite might have kept infection rates lower than would otherwise have occurred.

Analysis of sprouts, seeds, water, and environmental samples from sprouting facilities in both outbreaks failed to demonstrate *E. coli* O157 contamination. This is not surprising. A small percentage of seeds might be contaminated with *E. coli* O157. Because only a small portion of a lot is usually sampled, pathogen recovery is unlikely [17]. Two other studies that linked *E. coli* O157 outbreaks to alfalfa seeds failed to isolate this pathogen from implicated seeds [3, 17]. Moreover, in the Colorado investigation, there were no remaining seeds from the implicated lot to test.

Even if alfalfa seeds have low levels of contamination, the sprouting process can amplify bacterial contamination. Alfalfa sprouts in retail stores have been shown to contain up to 10^8–10^9 c.f.u. of bacteria per gram of sprouts [23]. Disinfecting sprouts after the sprouting process is ineffective because sprouts grown from contaminated seeds harbour pathogens in the inner tissues [24], and alfalfa sprouts are rarely cooked. Therefore, consumers may be directly exposed to pathogens contaminating sprouts if seeds are not decontaminated.

Considerable research has been directed at ways to decontaminate sprout seeds, including ozone, alcohol, NaOCl, $Ca(OCl)_2$, acidified $NaClO_2$, acidified ClO_2, Na_3PO_4, H_2O_2, heat, gamma irradiation [23], and electrolysed oxidizing water [25]. FDA guidelines recommend agitating alfalfa seeds in calcium hypochlorite (20 000 ppm) for 15 min [12], as the most effective chemical treatment available [26]. However, no chemical treatment has been shown to reliably decontaminate seeds. The Colorado outbreak occurred despite the sprouter's apparent compliance with FDA guidelines.

Two epidemiological studies suggest that FDA guidelines reduce risk from contaminated sprout seeds. In a 1999 multistate *Salmonella* outbreak, infection was linked to two sprouters that did not use chemical decontamination. Sprouters that used the same seed lots but applied appropriate decontamination procedures were not associated with the outbreak [19]. In a 1999 Colorado outbreak, *Salmonella* infection was linked to clover sprouts from a sprouter that followed FDA guidelines using calcium hypochlorite and agitation [27]. Infections linked to this sprouter occurred at a lower rate (0·29 cases/50 kg seed bag) than another sprouter in the same outbreak that did not follow FDA guidelines (1·13 cases/50 kg seed bag). Although these investigations suggested a benefit for FDA guidelines, they also indicated that guidelines are inconsistently followed and may not reliably eliminate sprout contamination.

Another FDA recommendation is to test spent irrigation water from each sprout production lot for *Salmonella* spp. and *E. coli* O157 [12]. The Minnesota sprouter failed to comply with this guideline. Inappropriate testing was previously associated with an outbreak of *Salmonella* Kottbus caused by contaminated alfalfa sprouts [28]. Hence, even if effective decontamination methods are devised, enforcement is required. No state or federal requirements currently exist for systematic inspections or certification of sprouting facilities [29].

Findings of this report are subject to at least three limitations. First, recall bias might have affected results. Patients were interviewed sooner after exposure than controls and potentially more motivated to identify exposures in order to identify the cause of illness. However, patients subjectively seemed focused on ground beef as the cause of illness. Second, only 46% of potential controls agreed to participate in the Colorado investigation, which might have resulted in a control group unrepresentative of the population from which cases originated. Against this, the 8% of controls that reported alfalfa sprout consumption was similar to 4·9% identified in the Colorado food survey. Third, no seed or environmental cultures corroborated the epidemiological evidence.

Minnesota's investigation revealed that enforcement of FDA guidelines for sprout seed decontamination and spent irrigation water testing are needed. Colorado's investigation suggested that even with compliance, current guidelines are insufficient to reliably ensure alfalfa sprout decontamination. Further research is needed. Unless cooked, which rarely occurs, alfalfa sprouts remain a potentially dangerous source of foodborne illness. Until reliable decontamination methods are devised, persons at high risk, e.g. the elderly, young children, and immunocompromised persons, should avoid raw sprout consumption [3, 30].

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