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High Incidence of Extra-Intestinal Infections in a *Salmonella Havana* Outbreak Associated with Alfalfa Sprouts

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SYNOPSIS

Objective. To determine a vehicle and point source for an outbreak of *Salmonella Havana*.

Methods. The authors conducted a case-control study and traceback investigation of 14 residents of California and four from Arizona with onsets of illness from April 15, 1998, to June 15, 1998, and *Salmonella Havana* infections with identical PFGE patterns.

Results. Seventeen of 18 patients were women. Seventeen were adults 20–89 years of age. Nine (50%) had diarrheal illness, 6 (33%) had urinary tract infections, 2 (11%) had sepsis, and one had an infected surgical wound after appendectomy. Four patients were hospitalized, and one died. Eating alfalfa sprouts was associated with *S. Havana* infection (OR = 10.0; 95% confidence interval 1.2, 83.1; $P = 0.01$).

Conclusions. This outbreak resulted in a high incidence of extra-intestinal infections, especially urinary tract infections, and high morbidity. Raw alfalfa sprouts, often considered a safe "health food," can be a source of serious foodborne disease outbreaks.

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Salmonella is one of the most common causes of foodborne bacterial infections in the United States. At least 70% of symptomatic infections involve enterocolitis with symptoms of diarrhea, fever, abdominal pain and cramps, nausea, and vomiting.[1,2] Some infections enter the blood (bacteremia) and result in a septicemic syndrome known as enteric fever or in a localized infection anywhere in the body. Bacteremia, morbidity, and mortality depend on a combination of host susceptibility and immunity, organism dose, and virulence characteristics of the organism.[3,4]

Because they are not sufficiently processed to destroy pathogenic microorganisms, raw dairy products[5,6] and unpasteurized apple juice[7] have caused outbreaks of *Salmonella* or *E. coli* O157:H7 infections. Some fresh fruits and vegetables that are eaten raw and are difficult to process, for example, lettuce[8] and raspberries,[9] have been linked to infection. Sprouts are another product eaten raw and are generally assumed safe, but contaminated sprouts have caused several disease outbreaks in the US and abroad.[10–14] Since 1995, the Centers for Disease Control and Prevention (CDC) have received information on more than 10 US outbreaks of *Salmonella* or *E. coli* O157:H7 infections associated with consumption of raw sprouts. In 1998, the California Department of Health Services (CDHS) investigated four outbreaks in California, including the *S. Havana* outbreak reported on here. The outbreak of *Salmonella Havana* infection associated with eating alfalfa sprouts involved significant morbidity and a high proportion of extra-intestinal infections.

METHODS

In late May 1998, the CDHS's Microbial Diseases Laboratory (MDL), which types all *Salmonella* isolates from the state of California, identified a cluster of more than 10 cases of H_2S-negative *Salmonella* serotype *Havana* infections. Fourteen cases were eventually identified during May and early June 1998.

Having established that one of the patients had traveled to Arizona during the week before onset of illness, the Disease Investigation Section of the CDHS contacted the Bureau of Epidemiology and Disease Control Services at the Arizona Department of Health Services to find out if its laboratory had found similar isolates. The Arizona laboratory reported to the CDHS that it had identified four cases of H2S-negative *S. Havana* infections among Arizona residents during May 1998.

The MDL confirmed all 18 *S. Havana* isolates with standard methods and further subtyped them using pulsed-field gel electrophoresis (PFGE), which provides a graphic electrophoresis "fingerprint" of the strain. The laboratory then compared this PFGE pattern among all 18 isolates and to prior strains of *S. Havana* maintained in a culture bank to determine whether this was a unique epidemic strain that likely came from a common source.

Identification of the common source. We defined cases as illnesses with *S. Havana* with onset from April 15, 1998, through June 15, 1998, with identical PFGE patterns. We then conducted a case-control study to identify risk factors.

Following extensive queries of six people who had been ill, epidemiologists from the Disease Investigation Section of the CDHS developed a standardized questionnaire that inquired about 20 food items. Each case patient identified as controls two acquaintances matched by county, sex, and 10-year age range. To enhance the power of the study, the investigators obtained two more controls using sequential telephone number dialing by adding or subtracting one digit from each case patient's telephone number. Epidemiologists and infection control nurses from local health departments conducted interviews of patients and controls by telephone from July 15 through August 1. We excluded patients from our analyses if they were unable to provide a food history or if investigators were unable to contact them during the study period.

The infection control nurses from the local health departments obtained clinical information from patients during the initial interview. For complicated cases or where the patient could not provide details, this information was supplemented by interviews with treating clinicians.

Statistical analysis. Present authors HB and JMB performed case-control matched analyses using Epi Info Version 6.04, public domain software available from the CDC. We calculated Mantel-Haenszel weighted odds ratios and 95% confidence intervals. Mantel-Haenszel summary chi squares provided estimates of statistical significance for two-tailed tests with $\alpha = 0.05$.

Traceback investigation. Once we determined through interviews that the food item associated with disease was alfalfa sprouts, we queried patients about where they had purchased or eaten sprouts, which in some cases required second telephone interviews. The Food and Drug Branch of the CDHS then reviewed invoices from these businesses to identified the producers of the sprouts. Next, they inspected the producers and reviewed records to

Sprouts, a food considered healthy by many consumers, can pose a risk for infectious disease.

identify which seed lots were sprouted and distributed to the restaurants and food outlets patronized by the case patients. Using invoice records, they tracked the seeds to the distributors and growers and investigated these businesses. A few months later while investigating an outbreak of *S. Cubana* associated with sprouts from this producer, investigators obtained seeds from one of the implicated seed lots to sprout and culture in the laboratory.

RESULTS

Descriptive epidemiology. Dates of onset of all 18 cases were clustered from April 29 to May 28, 1998. Patients ranged from 5 to 89 years (median age 46 years). (See Table.) Seventeen (94%) of the 18 patients were women. Four patients lived in southern Arizona. Fourteen patients lived in California; all but two of the 14 resided in southern California. One of the two patients who resided in northern California had been on vacation in Phoenix for the week before her illness.

Nine (50%) of the *S. Havana* isolates were obtained from stool, while the others were recovered from other sites: 6 from urine, 2 from blood (one from ascitic fluid as well), and one from a surgical wound. Clinical details were available for 16 (89%) of the 18 cases. No clinical details were available for a 15-year-old and a 30-year-old; both had *Salmonella* isolated from the stool and reported uncomplicated diarrhea to the investigators. Of the 7

Table. Patient characteristics and site of isolation of *Salmonella Havana*, California and Arizona outbreak, 1998

Age	*Sex*	*Date of onset of illness*	*Isolation site*	*Hospitalized*	*Significant previous medical problem*
32	F	April 26	Appendectomy wound	Yes	None
64	F	May 11	Blood/ascites	Yes	Cirrhosis
85	F	May 18[a]	Blood/stool	Yes (Died)	Diabetes
5	F	May 4	Stool	No	None
15	F	April 30[a]	Stool	No	No information
23	F	May 13	Stool	No	None
26	F	April 29	Stool	No	None
30	F	May 5	Stool	No	None
42	M	May 20[a]	Stool	No	HIV positive
50	F	May 1	Stool	No	None
59	F	April 30	Stool	No	None
75	F	May 2	Stool	No	None
25	F	May 26	Urine	No	None
26	F	May 8	Urine	No	First trimester pregnancy
71	F	May 10	Urine	No	Frequent cystitis, cardiac bypass
80	F	May 6	Urine	No	None
84	F	June 3[a]	Urine	Yes	Respiratory failure
89	F	May 28	Urine	No	Unspecified cardiovascular

[a]Date of specimen collection because date of onset was uncertain or unknown

Until safety can be assured, groups that are at high risk of morbidity from infection, especially the immunocompromised, young children, and older people, should avoid eating raw sprouts.

patients with *S. Havana* found in the stool for whom clinical information was available, all had diarrhea, 6 (87%) had fever, 6 (87%) had cramps, 5 (71%) had nausea, and 3 (43%) had bloody diarrhea.

The six patients with *S. Havana* isolated from their urine reported only symptoms of lower urinary tract infection (cystitis). The median age of patients with positive urine cultures was 76 years (range 25 to 89 years). Three were ages 80 years or older, but other than advanced age had no risk factors for *Salmonella* urinary tract infection, such as serious underlying illness, immunosuppression, or urinary tract abnormalities[1] (see Table). Of the five patients with urinary infections who were treated as outpatients, none reported significant diarrhea or had evidence of sepsis, according to their health practitioners, so neither stool cultures nor blood cultures were taken.

Four patients (22%) were hospitalized, and one died. A 32-year-old woman admitted to a hospital for suspected appendicitis had a normal appendix at surgery but developed a surgical wound infection from which *S. Havana* was unexpectedly cultured. Three of the hospitalizations, and the one death, occurred among elderly women ages 64–85 years (mean age 77 years). In each case, hospitalization was for decompensated chronic illness and a search for infection yielded *S. Havana*. An 85-year-old woman who had been discharged recently from the hospital to a nursing home after treatment for an *E. coli* urinary infection returned to the hospital because of poor control of her diabetes. *S. Havana* was isolated from her blood, and she died four days later. *S. Havana* was cultured from the urine of an 84-year-old woman hospitalized for respiratory failure. In the third case, *S. Havana* was cultured from both blood and ascitic fluid of a 64-year-old woman hospitalized for complications of cirrhosis and portal hypertension (abnormal increased blood pressure in the venous system of the liver).

Laboratory. All 18 *S. Havana* isolates, including those from residents of Arizona, were H_2S-negative. (H_2S gas can be detected by color change in selective media.) PFGE patterns were identical for all isolates from California and Arizona. H_2S-negative strains of *S. Havana* are quite rare and were not available in the MDL's microbial bank to test for heterogeneity. However, this PFGE pattern was different from those of two other strains of H_2S-positive *S. Havana* cultured at the MDL prior to this outbreak. (Heterogeneity with previous similar subtypes of *Salmonella* supports the uniqueness of the outbreak strain and suggests that cases in separate geographic areas are linked to the same point source.)

Of the *S. Havana* urine isolates identified by the MDL in 1986–1998, prior to the outbreak, 14/17 (82%) were from women, two were from men, and sex was unrecorded for one patient. The 13 women patients in our outbreak who resided in California were more likely to have isolates from urine (46%) than the 74 women with S. *Havana* infections in the MDL database prior to the outbreak (13.5%) (odds ration [OR] = 5.5; 95% confidence interval [CI] 1.3, 23.7; $P = 0.01$). The implication of this significant difference is unclear.

Case-control study. Twelve case patients and 42 control subjects responded to the questionnaire. We excluded six of the 18 case patients from all analyses: two could not be contacted; two were unavailable when the study was conducted, and two were unable to give a food history. For the analysis of each food item, we excluded patients who could not recall whether they had eaten that specific item.

Eating alfalfa sprouts was associated with *S. Havana* infection (OR = 10.0; 95% CI 1.2, 83.1; $P = 0.01$). No other food item was associated with infection. Eight (67%) of the 12 patients and 12 (29%) of the controls recalled eating sprouts. One additional patient who was interviewed after the study was completed said that she ate sprouts regularly and had likely ingested them during the week before her illness. Dose-response was not assessed because sprouts are generally eaten as a garnish on a sandwich or in a salad.

It is interesting to note the benefit of additional control subjects. The analysis with only acquaintance controls suggested that alfalfa sprouts was the likely food vehicle (OR undefined; 95% CI 1.05, ∞; $P = 0.07$). The random-dialed controls also implicated sprouts, but the confidence interval included 1 (OR 4.7; 95% CI 0.7, 32; $P = 0.05$). About 30% of the people in each control group said that they regularly ate sprouts.

Traceback investigation. Eight patients could recall where they had eaten or purchased sprouts in the week before onset of illness. One major regional producer of sprouts supplying Southern California and Arizona was the only source for five patients and one of multiple sources for two patients. The company's product distribution area matched the geographic areas of Southern California and Arizona in which cases were identified. A small proportion of this producer's product was distributed in the San Francisco Bay area, which could explain the one northern California patient who ate alfalfa sprouts in a sandwich purchased in the San Francisco Bay area the week before her illness. The 85-year-old Arizona woman who died was on a pureed diet that did not include sprouts; however, the hospital where she was treated one week prior to her diagnosis of *S. Havana* sepsis did use sprouts from the implicated sprout grower. Cross-contamination of her food via equipment or staff could account for her infection.

We obtained seeds from the California wholesaler that supplied the sprout grower, taken from the same lot that produced the sprouts implicated in this outbreak. These seeds yielded sprouts from which *S. Havana* was cultured. The PFGE pattern of this isolate matched the outbreak strain. The suspect sprout producer in this outbreak assured the Food and Drug Branch of the CDHS that they routinely followed the practice recommended by the US Food and Drug Administration and the International Sprout Growers Association to decontaminate seeds by soaking in a 2,000 parts per million (ppm) hypochlorite solution for 5 to 10 minutes.[15]

DISCUSSION

From 1986 through 1998, the MDL identified 6 to 24 temporally and geographically scattered human isolates of *S. Havana* per year (160 cases total). The unusual temporal and geographic clustering of patients in spring 1998 in Southern California led to the detection of an outbreak. An additional reason why the cases were suspected of representing a common source outbreak was that H_2S-negative isolates are very unusual; more than 95% of common *Salmonella* species produce H_2S gas.

This outbreak of *S. Havana* infections revealed an unusual pattern of morbidity and extra-intestinal infection. Four patients (22%) were hospitalized, and there was one death attributed to *Salmonella* sepsis complicated by chronic disease. Nine patients (50%) had extra-intestinal *Salmonella* infection of normally sterile fluids. Predictably, morbidity was high in the elderly patients, who had extra-intestinal *Salmonella* infections and chronic illnesses.

The young adult who was hospitalized for an appendectomy probably had *S. Havana* infection with symptoms that mimicked appendicitis, but the infection was only identified after *S. Havana* was cultured from a subsequent wound infection. *Salmonella* infection can produce appendicitis symptoms associated with mesenteric adenitis[16,17] or inflammation of the ileocecum.[18] However, *Salmonella* can also cause true appendicitis and peritonitis.[2,19] Wound infection leading to the initial diagnosis of *Salmonella* infection has also been noted following cholecystectomy.[1]

Other reports have suggested that *S. Havana* is highly virulent, but the clinical epidemiology may be more related to patient characteristics than to the organism. The serotype was first isolated in 1937 from spinal fluid following an outbreak of meningitis that resulted in 21 deaths among neonates in a hospital in Havana, Cuba.[2] Pavia et al. have suggested that *S. Havana* is a virulent strain that is more likely than other serotypes to cause bacteremia and extra-intestinal infection.[20] In Iran, where *S. Havana* is the second most common *Salmonella* serovar isolated from humans with diarrhea, Jafari et al. tested 132 isolates of *S. Havana* and found high levels of virulence as measured by adherence and invasiveness.[21] Of all 160 human isolates of *S. Havana* identified by the MDL over the past 13 years, 6 (3.7%) were from blood, compared with 11% in this series ($P = 0.16$). Our numbers were not sufficient to compare the relative virulence of our strain to the strains causing other S. *Havana* outbreaks or to other common *Salmonella* serotypes.

S. Havana was isolated from urine for 33% of patients in our outbreak. Previous reports describe urinary tract infections due to *Salmonellae* as a rare occurrence, except with enteric fever or other bacteremic forms of salmonellosis.[22,23] *Salmonella* urinary tract infections are associated with extremes of age, urologic abnormalities, surgery, and severe underlying disease.[1,4,24–26] Saphra and Winter reported that only 49 (0.52%) of 9,284 nontyphoidal *Salmonella* infections at the Mayo Clinic during 1948–1962

involved the urinary tract, primarily resulting in kidney infections; 3.2% of nontyphoidal *Salmonella* isolates were recovered from urine.[2] The experience of the California MDL is similar to that of the Mayo Clinic, with 3.4% of nearly 24,000 human isolates of *Salmonella* isolated from urine over a five-year period (1992–1996).[27]

The mechanism of spread to the urinary tract could be hematogenous or retrograde invasion by perineal flora. We found no literature to suggest why some serotypes may be more likely than others to cause ascending urinary tract infections. In our experience and as reported in earlier studies,[1,2,24] most patients with *Salmonella* isolated in the urine have symptoms of urinary tract infection, but few have concurrent or prior enteritis. Despite lack of diarrhea, Allenberger et al. found that 50% had positive stool cultures in addition to their urinary tract infections.[24] The urinary infections in our outbreak were most likely the result of ascending infections rather than hematogenous spread. Fever and other clinical signs of sepsis were absent and there was no severe underlying disease, immunosuppression, or known structural abnormality of the urinary tract in these patients. All had symptoms of urinary tract infection without diarrhea, but they could have had silent gastrointestinal infection. Our high incidence of urinary tract infections may be related in part to characteristics of the *S. Havana* serotype and in part to the predominance of adult women among our case patients. Women may be both more likely than men to eat sprouts and to have urinary tract infections. The implication of the high incidence of urinary infections among our female patients, even when compared to the incidence among prior *S. Havana* cases in our laboratory database, is unclear.

The descriptive epidemiology of our outbreak was similar to that seen in other sprout-associated outbreaks (Unpublished data, CDHS, 1996–1998).[11] Cases in these California outbreaks tended to be geographically widespread and did not involve other household members.[11] Patients tended to be adult women, whereas salmonellosis typically occurs at a higher rate in children than in adults and has equal sex distribution.[13]

Prevention of sprout-associated outbreaks of infections will entail a combination of approaches to protect seeds and sprouting equipment from contamination. The warm, moist conditions during sprouting are ideal for amplifying bacteria that may contaminate alfalfa seeds.[15,28] Sprout seeds are a raw agricultural product that is grown under the same conditions used for producing animal feed, including the use of manure instead of chemical fertilizer on some farms; seeds may become contaminated by vermin during production, storage, or distribution; and *Salmonellae* can persist for long periods on seeds. In Australia, *S. Havana* has been found in several non-human sources including wild birds and poultry[29] and is reportedly the most frequently isolated *Salmonella* serotype from animal feed, accounting for 24% of *Salmonella*-contaminated samples.[30]

In late 1998, the CDHS recommended increasing the concentration of hypochlorite in water used to soak sprout seeds for decontamination from 2,000 ppm to 20,000 ppm. Recent work has suggested that the lower concentration is inadequate to eliminate *Salmonella* and *E. coli* O157 from alfalfa seed[31] and that the higher concentration is more effective, but still may not kill all pathogenic bacteria.[32] Other practices and policies to assure microbiologic safety are being evaluated by agricultural and food researchers.

Conclusions. This outbreak demonstrated an unusually high incidence of urinary tract infections causing cystitis in patients with no diarrhea and no underlying abnormalities of the urinary tract. Extra-intestinal infection may not be synonymous with invasive infection, and ascending infections after bowel contamination is the most likely mechanism for the urinary tract infections.

Epidemiology, product tracing, and laboratory results linked all these cases to contaminated alfalfa sprout seeds. Sprouts, a food considered healthy by many consumers, can pose a risk for infectious disease. Until safety can be assured, groups that are at high risk of morbidity from infection, especially the immunocompromised, young children, and older people, should avoid eating them raw.[14] All consumers should be advised of the risk of eating alfalfa sprouts through product labeling, press releases,[33] and other publicity efforts so that they can make an informed decision whether to eat them.

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