Multistate Outbreak of *Salmonella* Typhimurium and Saintpaul Infections Associated with Unpasteurized Orange Juice—United States, 2005

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Background. Infection due to *Salmonella* species causes an estimated 1.4 million illnesses and 400 deaths annually in the United States. Orange juice is a known vehicle of salmonellosis, for which regulatory controls have recently been implemented. We investigated a nationwide outbreak of *Salmonella* infection to determine the magnitude of the outbreak and to identify risk factors for infection.

Methods. We identified cases through national laboratory-based surveillance. In a case-control study, we defined a case as infection with *Salmonella* serotype Typhimurium that demonstrated the outbreak pulsed-field gel electrophoresis pattern in a person with illness onset from 1 May through 31 July 2005; control subjects were identified through random digit dialing.

Results. We identified 152 cases in 23 states. Detailed information was available for 95 cases. The median age of patients was 23 years; 46 (48%) of the 95 patients were female. For 38 patients and 53 age-group matched control subjects in 5 states, illness was associated with consuming orange juice (90% vs. 43%; odds ratio, 22.2; 95% confidence interval, 3.5–927.5). In a conditional logistic regression model, illness was associated with consuming unpasteurized orange juice from company X (53% vs. 0%; odds ratio, 38.0; 95% confidence interval, 6.5–infinity). The US Food and Drug Administration found that company X was noncompliant with the juice Hazard Analysis and Critical Control Point regulation and isolated *Salmonella* serotype Saintpaul from company X's orange juice.

Conclusions. Unpasteurized orange juice from company X was the vehicle of a widespread outbreak of salmonellosis. Although the route of contamination is unknown, noncompliance with the juice Hazard Analysis and Critical Control Point regulation likely contributed to this outbreak. Pasteurization or other reliable treatment of orange juice could prevent similar outbreaks.

Infection due to *Salmonella* species causes an estimated 1.4 million illnesses and 400 deaths annually in the United States [1]. Contaminated fruits and vegetables, including juices, are increasingly recognized sources of outbreaks of *Salmonella* infection [2]. From 1995 through 2004, there were 19 juice-associated outbreaks reported to the Centers for Disease Control and Prevention (CDC); 7 (37%) were associated with orange juice [3]. Orange juice is commonly consumed: 33% of a US population sample reported drinking orange juice in a given week in 2002 [4]. To address factors that contributed to juice-associated outbreaks, the US Food and Drug Administration (FDA) introduced the juice Hazard Analysis and Critical Control Point (HACCP) regulation for the safe and sanitary processing of juice in 2001; it was fully implemented by 2004. We describe the first reported juice outbreak since the juice HACCP regulation's full implementation.

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OUTBREAK

In June and July 2005, the Michigan Department of Community Health investigated 14 cases of illness due to *Salmonella* serotype Typhimurium with indistinguishable PFGE patterns by 2 restriction enzymes. This outbreak strain was new to the database in PulseNet, the national molecular subtyping network for foodborne pathogens [5]. Eleven of 13 interviewed patients reported consuming store brand "fresh-squeezed" orange juice from 1 of 2 upscale market retailers. Investigation by the Michigan Department of Agriculture revealed that the orange juice identified by patients was supplied to both retailers by 1 juice processor in Florida, referred to here as company X.

By 1 July 2005, we identified 45 cases caused by the outbreak strain of *S.* Typhimurium in 6 states. We conducted a multistate investigation to determine the magnitude of the outbreak, to identify risk factors for *S.* Typhimurium infection, and to recommend infection-control measures. After an FDA report on 27 July 2005 that testing of orange juice samples from company X had yielded *Salmonella* serotype Saintpaul, case-finding and collection of exposure data was expanded to include patients with infection due to *S.* Saintpaul.

PATIENTS, MATERIALS, AND METHODS

Case-finding. We defined an *S.* Typhimurium case as infection with *S.* Typhimurium that demonstrated PulseNet PFGE *Xba*I pattern JPX001.0178 and *Bln*I pattern JPXA26.0186 (the outbreak strain patterns) in a person with illness onset from 1 May through 31 July 2005. We defined an *S.* Saintpaul case as infection with *S.* Saintpaul that demonstrated PulseNet *Xba*I pattern JN6X01.0030 (same as the FDA juice sample) in a person with illness onset from 1 May through 31 July 2005. We requested that state public health laboratories perform PFGE analysis on all *S.* Typhimurium and *S.* Saintpaul isolates received from 1 May through 6 December 2005, and we queried the PulseNet database for matching outbreak isolates. We posted messages to an email listserv that included federal, state, and local foodborne disease investigators and Epi-X, the Epidemic Information Exchange system, requesting that health departments report outbreak-related cases and interview patients by means of a standardized questionnaire.

To formulate hypotheses with regard to possible food vehicles, we compared preliminary case exposure findings in Michigan with those in Maine, Massachusetts, New Hampshire, Ohio, and Pennsylvania. Retailers were contacted to determine the source of their orange juice.

Case-control study. For the case-control study, we defined a case as infection with the outbreak strain of *S.* Typhimurium in a person with illness onset from 1 May 2005 through the time of their interview. Control subjects were persons who had not had diarrhea since 15 May 2005; control subjects were identified through random digit dialing generated by patient telephone number exchange and were matched by age group (1–5 years, 6–17 years, 18–50 years, and >50 years). For all study participants aged <18 years, guardians were interviewed.

State and CDC officials conducted the case-control study from 8 July through 16 July 2005. A questionnaire was administered to patients with outbreak-related cases who were identified after 16 July, but control subjects were not obtained to correspond to these patients.

Hypothesis-generating interviews identified commonly consumed foods and aided development of the questionnaire that addressed juice consumption during the 7 days before illness onset. The control exposure period was the first week of June 2005. To obtain detailed juice exposure information, we developed a supplemental questionnaire for state and local health officials to record the brand name, store name, and location of purchase (obtained from the patient), and the name and location of the orange juice distributor (obtained from store personnel). If possible, health officials contacted distributors directly to determine the source of their product.

Trace-back and environmental investigation. State and local health officials investigated venues where company X orange juice was purchased. FDA officials inspected company X's facilities and collected maintenance and microbiologic records, environmental swab samples, and orange juice samples.

Laboratory investigation. *Salmonella* isolates were serotyped and subtyped by PFGE at state public health laboratories. Select isolates of the *S.* Typhimurium outbreak strain were confirmed at the CDC, and antimicrobial susceptibility testing was performed on 4 isolates through the CDC's National Antimicrobial Resistance Monitoring System for Enteric Bacteria. Samples of leftover company X orange juice were collected from patients and establishments from 11 July through 1 August 2005 and were cultured for bacteria at the CDC or the respective state public health laboratories, according to procedures outlined in the FDA's *Bacteriological Analytical Manual* [6]. Juice and environmental samples obtained during the FDA's investigation of company X were tested at FDA laboratories.

Statistical analysis. Statistical analyses were conducted using SAS, version 9.1 (SAS Institute). We used bivariable and multivariable conditional logistic regression models to investigate associations with disease. We report odds ratios (ORs) with exact 95% confidence intervals (CIs) on the basis of these models for each categorical variable. Likelihood-ratio tests were used to assess multivariable models. All exposures with significant associations, substantial case exposure, and biological plausibility were examined.

RESULTS

Case-finding. We identified 152 cases of *S.* Typhimurium infection in 23 states (figure 1); detailed information was available

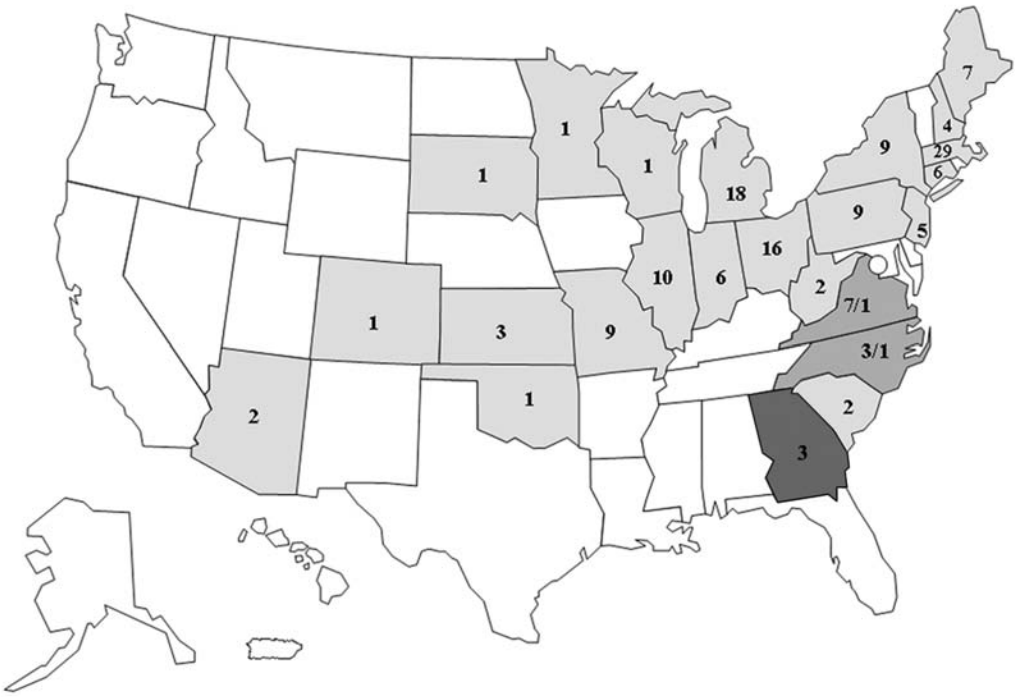

Figure 1. Outbreak cases of infection, by state, 2005 ($N = 157$). *Light gray,* all cases due to *Salmonella* serotype Typhimurium; *dark gray,* all cases due to *Salmonella* serotype Saintpaul; *medium gray,* cases due to both pathogens (the first numeral represents cases due to *S.* Typhimurium).

for 95 cases (63%). Dates of illness onset ranged from 11 May through 4 July 2005, with most cases occurring from 27 May through 14 June 2005 (figure 2). The median age of these patients was 23 years (range, 6 months–78 years); 46 (48%) were female. The median duration of illness was 7 days (range, 2–23 days). There were no reported deaths; 89 (94%) of patients sought medical care, and 23 (24%) were hospitalized (table 1).

During the outbreak period, 5 cases of *S.* Saintpaul infection were detected in 3 states (figure 1). Dates of illness onset ranged from 29 June through 13 July 2005 (figure 2). The median age of these patients was 5 years (range, 4–7 years); 1 was female. None were hospitalized, and none died.

Of the 95 patients with *S.* Typhimurium infection for whom detailed information was available, 73 (77%) drank orange juice during the week before illness. Of these 73 patients, orange juice brand information was available for 72 (99%); of these 72 patients, 39 (54%) from 13 states drank company X orange juice. Of the 4 patients with *S.* Saintpaul infection for whom detailed information was available, 3 drank orange juice during the week before illness, 1 of whom drank company X orange juice.

Case-control study. We enrolled 38 patients with *S.* Typhimurium infection and 53 control subjects from 5 states (Maine, Massachusetts, Michigan, Ohio, and Pennsylvania). On bivariable analysis, consumption of orange juice, reported by 34 (89%) of 38 patients and 23 (43%) of 53 control subjects, was strongly associated with illness (OR, 22.2; 95% CI, 3.5–927.5) (table 2). No other food or beverage was associated with illness.

Patients were more likely than control subjects to have consumed orange juice from company X (OR, 41.0; 95% CI, 7.1 to ∞) or company Y (OR, 4.2; 95% CI, 1.1–24.2). Case exposure for company X orange juice was 53% and for company Y was 37%. No other orange juice brand was consumed by >6% of patients. In a conditional logistic regression model, including variables for consumption of orange juice from company X and company Y, only company X orange juice remained significantly associated with illness (OR, 38.0; 95% CI, 6.5 to ∞).

Trace-back and environmental investigation. On 8 July 2005, on the basis of preliminary epidemiologic information, the FDA issued a nationwide warning against drinking unpasteurized orange juice products distributed under a variety of brand names by company X [7]. On 9 July 2005, company X voluntarily recalled all of its unpasteurized orange juice in commerce and certain lots of frozen unpasteurized product, both

sold under many different labels, and initiated flash pasteurization of its orange juice (figure 2) [8].

Orange juice from company X was produced in 1 plant. Company X received oranges in 3 ways: directly from orchards; from packing houses' cold storage, where fruit was sorted, damaged fruit was culled, and the remaining fruit was washed and waxed; or from the company's own cold storage where damaged fruit was not culled, nor was remaining fruit washed and waxed. Company X's citrus fruit underwent surface treatment; the details of the treatment were not described but included washing with water and a sanitizer, washing with a brush, and steaming. After juice was extracted, it was immediately chilled to −2.2°C to −1.1°C and shipped for delivery within 72 h; the final product had a 12–22-day shelf life, depending on storage temperature [9]. Company X orange juice was labeled as "all natural fresh-squeezed" without an indication that the final juice product was not pasteurized or was not treated with any method to reduce pathogens [7]. Company X distributed unpasteurized refrigerated orange juice and unpasteurized frozen orange juice to 31 states, Puerto Rico, Canada, France, and Japan [8].

From 11 July through 28 July 2005, the FDA investigated company X's facilities and found that company X was noncompliant with the juice HACCP regulation at several points [10]. From January through July 2005, company X's quality-control tests on its fresh-squeezed juice were repeatedly positive

Table 1. Clinical characteristics of patients infected with the outbreak strains of *Salmonella* serotype Typhimurium and *Salmonella* serotype Saintpaul.

Characteristic	*S.* Typhimurium (n = 95)	*S.* Saintpaul (n = 4)
Symptom		
Diarrhea	91 (96)	4 (100)
Abdominal cramps	85 (89)	4 (100)
Fever	77 (81)	3 (75)
Blood in stool	53 (56)	0 (0)
Headache	52 (55)	2 (50)
Nausea	42 (44)	1 (25)
Vomiting	35 (37)	1 (25)
Sought medical care	89 (94)	2 (50)
Received antibiotics	55 (58)	2 (50)
Missed work or school	44 (46)	1 (25)
Hospitalized	23 (24)	0 (0)

NOTE. No patients died.

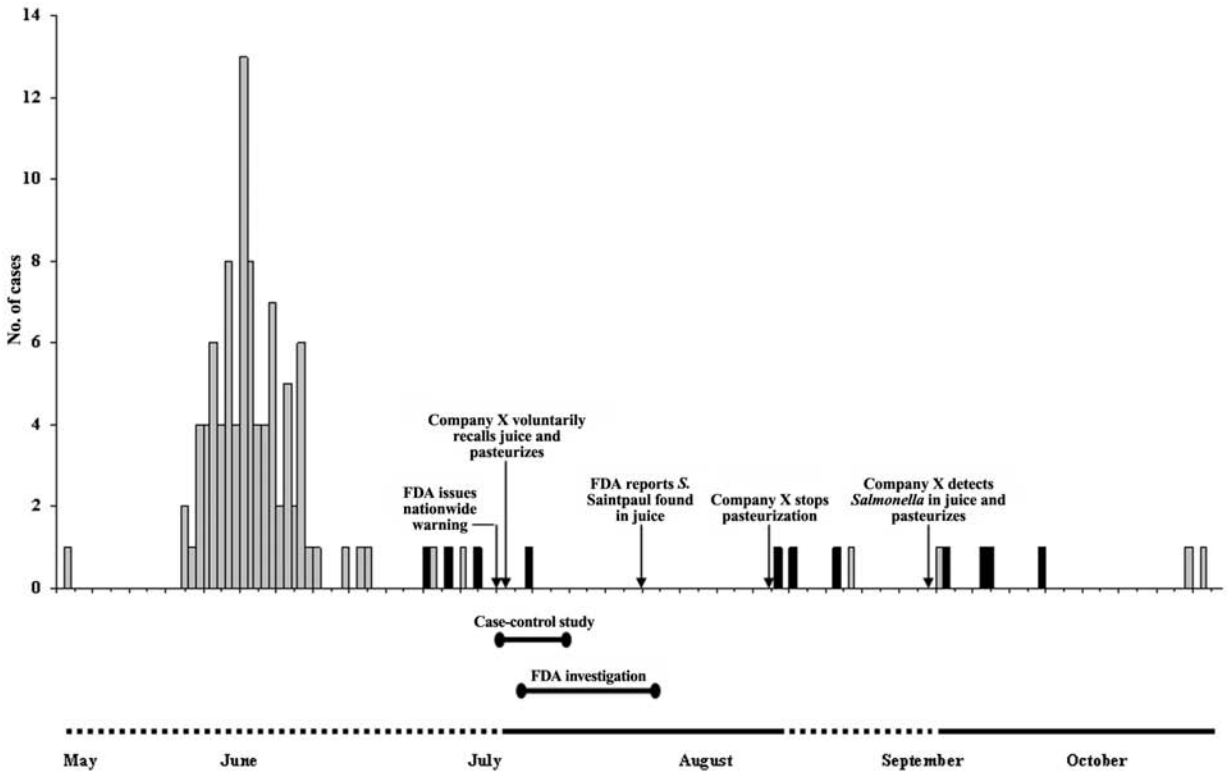

Figure 2. Number of cases of infection, by date of illness onset, and time line of investigation events, in a multistate outbreak of *Salmonella* infection—United States, 2005. Cases reported after 13 July are listed by the date that a *Salmonella* isolate was found in a stool sample. *Gray bars,* cases due to *Salmonella* serotype Typhimurium; *black bars,* cases due to *Salmonella* serotype Saintpaul. The solid and dotted line below the x-axis indicates periods when company X sold pasteurized *(solid line)* or unpasteurized *(dotted line)* orange juice.

Table 2. Bivariable analysis of selected exposures among patients with infection due to *Salmonella* serotype Typhimurium and age-group matched control subjects.

Exposure	Proportion of patients (%) (n = 38)	Proportion of control subjects (%) (n = 53)	Estimated OR (95% CI)[a]
Orange juice[b]	34/38 (89)	23/53 (43)	22.2 (3.5–927.5)
At a restaurant	15/26 (58)	1/21 (5)	7.0 (0.9–315.5)
At home	29/33 (88)	22/23 (96)	1.0 (0.03 to ∞)[c]
"Fresh-squeezed"	12/21 (57)	2/19 (11)	4.7 (0.5–236.3)
Not made from concentrate	15/21 (71)	11/21 (52)	2.6 (0.4–27.7)
Orange juice from company X	20/38 (53)	0/53 (0)	41.0 (7.1 to ∞)[c]
Orange juice from company Y	14/38 (37)	10/53 (19)	4.2 (1.1–24.2)
Travel	14/37 (38)	7/52 (14)	4.0 (1.2-17.3)
Lettuce	30/34 (88)	36/50 (72)	3.0 (0.8–17.6)
Apple juice	8/32 (25)	9/53 (17)	2.3 (0.6–9.0)
Grape juice	16/27 (59)	30/52 (58)	1.2 (0.3–5.3)
Tomato juice	22/32 (69)	35/52 (67)	1.0 (0.3–3.7)

[a] Point estimates are maximum likelihood unless otherwise indicated. The likelihood is the exact conditional likelihood and, thus, 95% CIs are exact.
[b] Denominators vary because of missing information.
[c] This OR is a median unbiased estimate.

for generic *Escherichia coli*. During this period, company X did not use an alternate method to process their juice as required by the juice HACCP regulation. In addition, company X did not maintain the required sanitation monitoring records and used substandard procedures for cleaning equipment between different citrus juice processing operations.

On 15 August 2005, company X reinitiated shipment of its fresh-squeezed unpasteurized citrus juices and introduced a new "gourmet pasteurized" line of citrus juices. On 6 September 2005, company X voluntarily recalled and ceased production of its fresh-squeezed unpasteurized orange juice and reinstituted flash pasteurization of its products, after routine internal testing indicated possible contamination with *Salmonella* species (figure 2) [11]. To date, company X produces both "fresh-squeezed" (unpasteurized) and "gourmet pasteurized" orange juice.

Laboratory investigation. From 1 May through 31 July 2005, 152 *S.* Typhimurium isolates were identified as part of this outbreak. Four additional *S.* Typhimurium isolates were identified from 26 August through 13 October; no additional isolates were identified during the surveillance period, which ended 6 December 2005 (figure 2). Sources of the *S.* Typhimurium isolates were samples of stool (n = 149), urine (n = 5), or blood (n = 2). From 1996 until the time this outbreak was first detected in June 2005, the outbreak strain of *S.* Typhimurium had never previously been identified among 23,129 *S.* Typhimurium isolates in the PulseNet database. At the National Antimicrobial Resistance Monitoring System for Enteric Bacteria laboratory, 4 isolates of the *S.* Typhimurium outbreak strain were susceptible to the 15 antimicrobial agents tested, which were as follows: amikacin, amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole.

From 1 May through 31 July 2005, there were 5 *S.* Saintpaul isolates identified as part of this outbreak. Seven additional isolates that matched the pattern were identified from 16 August through 21 September; no additional isolates were identified during the surveillance period, which ended 6 December 2005 (figure 2). The source of all 12 *S.* Saintpaul isolates was stool samples. From 1996 until the time this outbreak was first detected in June 2005, this *Xba*l pattern accounted for 1% of the 1292 total *S.* Saintpaul isolates in the PulseNet database.

None of the 4 orange juice samples from patients or establishments with leftover company X orange juice yielded *Salmonella* species (table 3). FDA testing of unpasteurized orange juice from an unopened 1-pint plastic jug labeled with a "use by" date of 25 July 2005 and obtained during their company X facility investigation yielded *S.* Saintpaul (PulseNet *Xba*1 pattern JN6X01.0030).

DISCUSSION

A nationwide outbreak of infection due to *S.* Typhimurium and *S.* Saintpaul from May through July 2005 was associated with unpasteurized orange juice manufactured by company X. To our knowledge, this is the first reported outbreak associated with orange juice since full implementation of the juice HACCP regulation in 2004. State public health agencies and the CDC

Table 3. Data on laboratory analysis of samples of company X's unpasteurized orange juice.

State of origin	Provider of sample	Laboratory method, location	Date received	Description of orange juice	Results
Connecticut	Establishment	FDA BAM, state public health lab	14 July 2005	"Use by" 17 July 2005; 1 gal (3.8 L)	*Klebsiella pneumoniae*
Maine	Establishment	FDA BAM, state public health lab	19 July 2005	"Use by" 17 July 2005; 2 gal (7.6 L)	Negative
Ohio	Patient	Culture, CDC	12 July 2005	"Use by" 17 July 2005; half gal (1.9 L)	Negative
Virginia	Patient	Culture, CDC	2 August 2005	Bought on 22 June 2005; half gal (1.9 L)	Negative
Florida	Establishment	FDA BAM, FDA	14 July 2005	"Use by" 25 July 2005; 1 pt (0.47 L)	*Salmonella* serotype Saintpaul

Note. All samples were from unopened containers. CDC, Centers for Disease Control and Prevention; FDA BAM, US Food and Drug Administration method based on *Bacteriological Analytical Manual*.

identified 157 cases in 24 states; however, if we take underreporting into account [1, 12], we estimate that 6000 persons may have become ill. After the outbreak period, continued surveillance through December 2005 identified 4 cases of infection due to *S.* Typhimurium and 7 cases of infection due to *S.* Saintpaul that matched the outbreak patterns. Although we had insufficient information to determine if these patients had consumed company X orange juice, the exposure periods for 2 *S.* Typhimurium and 6 *S.* Saintpaul cases occurred during the time that company X continued to distribute unpasteurized orange juice.

Routine subtyping of *Salmonella* isolates by serotype and PFGE pattern and ascertainment of specific food exposure information—not only by brand name, but also by manufacturer—were key to determining the cause of the outbreak. Although *S.* Typhimurium is the most common *Salmonella* serotype isolated in the United States, further differentiation by PFGE allowed us to create a very specific case definition for the analytic study. Because the contaminated orange juice was sold under many different brand names and was consumed by the glass in restaurants, detailed juice source information obtained from restaurant and store personnel was imperative to confirm an association with company X orange juice. Furthermore, 5 patients with *S.* Saintpaul infection were linked to company X orange juice by the FDA juice sample yielding a strain with the same PFGE pattern and by detailed exposure information for 1 of those patients.

Lessons learned from previous juice-associated outbreaks helped shape the current juice HACCP regulation. In 1999, there was an outbreak of infection due to *Salmonella* serotype Muenchen, with 207 cases reported from 15 US states and 2 Canadian provinces; illness was associated with consumption of commercially distributed unpasteurized orange juice that had undergone a pathogen-reduction process thought to be equivalent to pasteurization [13]. In 2000, an outbreak of infection due to *Salmonella* serotype Enteritidis was reported from 10 states; illness was associated with consumption of unpasteurized, commercial juice that was squeezed after a sanitizer was applied to the fruit's surface—a processing method that was considered to be acceptable at that time [14].

The juice HACCP regulation requires that all juice processors, except retail establishments, apply control measures capable of achieving a 100,000-fold (5-log) pathogen reduction in the number of microorganisms of interest to public health (usually *E. coli* or *Salmonella* species). Juice processors can use 1 control measure shown to reduce the number of pertinent microorganisms by at least 5 logs or a combination of control measures that have a cumulative effect of a 5-log pathogen reduction. Citrus juice processors, such as company X, may use surface treatment of the fruit to contribute to the 5-log reduction. Processors of all other juice types must apply the 5-log process to the juice itself [15]. Companies are not required to use methods approved by the FDA but are required to internally validate their processes. When process control indicators demonstrate that a validated process is not working as designed, the juice HACCP regulation requires corrective action. If the juice results from a validated process, the lack of heat treatment (i.e., pasteurization) does not have to be indicated on the label. Compliance with the juice HACCP regulation is considered to be the responsibility of the juice processor.

It is unknown how the orange juice in this outbreak became contaminated. Fruit may have become contaminated in the orchard, and *Salmonella* species on the fruit's surface may have survived treatment and subsequently contaminated the juice. Alternatively, *Salmonella* species could have been internalized if the fruit was damaged, in which case external fruit washing would be of no benefit. Further research is needed to better understand how contamination occurs, especially if juice continues to be produced by methods other than heat pasteurization of the final product.

This investigation is subject to limitations. The small number of cases of *S.* Saintpaul infection precluded making an epidemiological association with company X orange juice. However, company X orange juice consumption for even 1 of 4 patients with this infection was more than expected, given the low prevalence of company X orange juice consumption in the population (i.e., it was consumed by none of the 53 control subjects). Because information on the juice source from both patients and establishments was limited, exposure information with regard to manufacturer may have been incomplete. Although *S.* Typhimurium was not recovered in the leftover juice samples collected from patients and establishments or during the FDA's inspection, these convenience samples are not likely to be rep-

resentative of the same lots of juice actually consumed by the corresponding patients, given the time lag from onset of infection to juice sample collection. There were 42 days from the time that the second patient became ill on 27 May (when an outbreak could be considered to have started) and the time that the implicated product was removed from commerce on 9 July. Although detection of outbreaks of foodborne illness has improved greatly through laboratory-based molecular subtyping surveillance, delays in investigation and public health action, some inherent in the logistics of food distribution and the biology of foodborne infections, still exist. Efforts to improve public health system efficiency are ongoing.

Juice safety has improved, partly because of measures taken in response to problems identified through outbreak investigations, but several gaps remain in the system [3]. Although most juice producers have opted for pasteurization, some rely on other methods for pathogen reduction, as allowed under the current juice HACCP regulation. Because company X was not compliant with the juice HACCP regulation, we are unable to determine whether the regulation itself is deficient. Nevertheless, this national outbreak indicates that the problem of salmonellosis associated with contaminated juice has not been resolved. Continued close regulatory oversight of fresh citrus juice producers is needed to prevent additional illnesses. This oversight includes an assessment of whether fresh citrus juice processors are in compliance with the juice HACCP regulation and education and guidance for juice producers to ensure diligent adherence to the provisions of the regulation. To help ensure public health and food safety, further research is needed to understand mechanisms of juice contamination and the effectiveness of pathogen-reduction methods for juice. Juice labeling that indicates the pathogen-reduction method used might improve consumer awareness and should be considered. Pasteurization or other reliable treatment of orange juice may prevent subsequent outbreaks.

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