MAJOR ARTICLE

A Multistate Outbreak of *Salmonella enterica* Serotype Newport Infection Linked to Mango Consumption: Impact of Water-Dip Disinfestation Technology

Sumathi Sivapalasingam,[1,2] E. Barrett,[4] A. Kimura,[5] S. Van Duyne,[1] W. De Witt,[1] M. Ying,[1] A. Frisch,[1] Q. Phan,[6] E. Gould,[7] P. Shillam,[8] V. Reddy,[9] T. Cooper,[10] M. Hoekstra,[3] C. Higgins,[1] J. P. Sanders,[11] R. V. Tauxe,[1] and L. Slutsker[1]

[1]Foodborne and Diarrheal Diseases Branch, National Center for Infectious Diseases, National Center for Environmental Health, [2]Epidemic Intelligence Service, Epidemiology Program Office, and [3]Biostatistics and Information Management Branch, Centers for Disease Control and Prevention, Atlanta, Georgia; [4]Virginia Department of Health, Richmond; [5]California Department of Health Services, Gardena; [6]Connecticut Department of Health, Hartford; [7]Massachusetts Department of Health, Jamaica Plain; [8]Colorado Department of Health, Denver; [9]New York City Department of Health and Mental Hygiene, New York; [10]Rhode Island Department of Health, Providence; and [11]US Food and Drug Administration, Rockville, Maryland

(See the editorial commentary Jones and Schaffner on pages 1591–2)

Fresh produce increasingly is recognized as an important source of salmonellosis in the United States. In December 1999, the Centers for Disease Control and Prevention detected a nationwide increase in *Salmonella* serotype Newport (SN) infections that had occurred during the previous month. SN isolates recovered from patients in this cluster had indistinguishable pulsed-field gel electrophoresis (PFGE) patterns (which identified the outbreak strain), suggesting a common source. Seventy-eight patients from 13 states were infected with the outbreak strain. Fifteen patients were hospitalized; 2 died. Among 28 patients enrolled in the matched case-control study, 14 (50%) reported they ate mangoes in the 5 days before illness onset, compared with 4 (10%) of the control subjects during the same period (matched odds ratio, 21.6; 95% confidence interval, 3.53–∞; $P = .0001$). Traceback of the implicated mangoes led to a single Brazilian farm, where we identified hot water treatment as a possible point of contamination; this is a relatively new process to prevent importation of an agricultural pest, the Mediterranean fruit fly. This is the first reported outbreak of salmonellosis implicating mangoes. PFGE was critical to the timely recognition of this nationwide outbreak. This outbreak highlights the potential global health impact of foodborne diseases and newly implemented food processes.

Americans are eating more fruits and vegetables, encouraged by evidence of their beneficial role in health and nutrition [1]. The increased demand for produce in the United States has been met by a greater availability of these products, regardless of season, partly through importation. The international trade efforts include preventing inadvertent transport of agricultural pests, such as the Mediterranean fruit fly.

Concurrently, the number of produce-associated outbreaks reported annually in the United States has been increasing, from 4 during 1973–1987 to 10 during 1988–1991[2]. Detecting and investigating produce-associated outbreaks is more difficult than for outbreaks involving other foods because the wide distribution of produce results in geographically dispersed outbreaks with low attack rates. Two new public health tools have been developed to aid in the detection and investigation of such outbreaks. The Salmonella Outbreak Detection Algorithm (SODA) is a computer algorithm that can flag significant increases in the frequency of specific *Salmonella* serotypes reported to the Centers for Disease Control and Prevention (CDC; Atlanta) by state public health laboratories [3]. The second tool is PulseNet, a national network of public health laboratories that per-

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Reprints or correspondence: Dr. Robert V. Tauxe, Foodborne and Diarrheal Branch, Centers for Disease Control and Prevention, 1600 Clifton Rd., MS A-38, Atlanta, GA 30030 (rvt1@cdc.gov).

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form PFGE on foodborne bacteria and share information within the network to detect clusters of isolates with indistinguishable patterns, suggesting the occurrence of an outbreak from a single source [4].

These tools were useful in the detection and investigation of a recent outbreak of illness due to *Salmonella enterica* serotype Newport (SN) linked to consumption of imported mangoes. This outbreak illustrates the global nature of our food supply, particularly produce, and how a method used to prevent importation of agricultural pests in fruit can result in inadvertent contamination with human pathogens, as well as why food processing conditions in other countries are now of immediate relevance to the American consumer.

METHODS

The outbreak. In January 2000, public health officials in Virginia noted that PFGE patterns of SN isolates recovered from 5 patients who were ill during November and December of 1999 were indistinguishable (this identified the outbreak pattern). Because PFGE patterns of SN are not generally homogeneous [5], this cluster of an unusual pattern suggested that an outbreak might be occurring. An electronic image of the outbreak pattern was sent by e-mail to health departments in other states and to the CDC for comparison. At the same time, SODA identified a significant national increase in the prevalence of SN reported during November. On 11 January 2000, the CDC requested that PFGE be performed for all SN isolates received at state laboratories after 1 November 1999 and that results be submitted electronically to the PulseNet database. To determine whether there was an outbreak of SN infection in Europe, information was shared with public health officials at Enter-Net, an international system for laboratory-based *Salmonella* (and other enteric organisms) surveillance in all 15 European Union members; Enter-Net also uses a statistical algorithm to detect increases.

Epidemiologic investigation. A case was defined as diarrhea with onset occurring during the period of 1 November through 31 December 1999 for which a stool culture yielded SN with the outbreak PFGE pattern. We conducted open-ended hypothesis-generating telephone interviews with case patients to assess common exposures. This revealed that many case patients were Asian or Latino. Therefore, 2 control subjects were matched to each case patient by race/ethnicity and age in the case-control study. The age group categories were 0–5 months, 6 months to 1 year, 2–5 years, 6–11 years, 12–19 years, 20–50 years, and ⩾51 years. Patients who were unreachable by telephone after 3 separate attempts were not enrolled in the study. Control subjects were identified by asking the case patient to name 2 friends or coworkers of the same age group and ethnicity who did not live or share meals with them. Ethnicity was self-defined. We excluded potential control subjects who had diarrhea, fever, or gastrointestinal illness after 1 November 1999 or who had traveled abroad in the 5 days before onset of illness in the case patient.

Using a written questionnaire, we interviewed patients and control subjects about demographic information, course of their illness (for case patients), food consumption in the 5 days before the date of the case patient's illness onset, and location of purchase of selected food items. Spanish-speaking patients were interviewed in Spanish.

Traceback and environmental investigations. Traceback was conducted by the US Food and Drug Administration (FDA; Rockville, MD) using information on venue locations where case patients reported purchasing or eating mangoes during the 5 days before illness onset. Only patients who recalled purchasing mangoes from a single venue on a single day were considered for the traceback investigation. Of these, only those who remembered the name and address of the venue and date of purchase or who had a receipt of purchase for mangoes eaten during the 5 days before illness onset were included in the traceback investigation.

The FDA, the US Department of Agriculture (USDA)/Animal Plant Health Inspection Service (APHIS), and the CDC conducted an investigation of the implicated farm. Personnel were interviewed regarding worker health, farming, processing, and transport practices. Seven 1-L water samples were obtained from source and stored water. Cloacal samples were obtained from a toad found within 5 m of the processing tanks.

Laboratory investigation. *Salmonella* isolates were submitted from clinical laboratories to state public health laboratories for routine serotyping. Molecular subtyping of SN isolates was performed using standard methods for PFGE [4, 6]. Antimicrobial resistance testing of 18 isolates was performed at the CDC using the broth microdilution technique (Sensititre; Trek Diagnostics) using a standard panel of antimicrobial agents (apramycin, amikacin, ampicillin, amoxicillin–clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole) [7, 8].

Water samples were tested for fecal coliforms and *Salmonella* at the Recife Veterinary Diagnostic Laboratory (Recife, Brazil). The most probable number (MPN) of *Escherichia coli* and the detection and classification of *Salmonella* from water samples were determined using standard methods [9–11]. Biochemical confirmation of *Salmonella* was conducted using the API 20E kit (bioMérieux). Toad cloacal samples were transported in Cary-Blair medium (Difco Laboratories) to the CDC for *Salmonella* isolation and serotyping using standard techniques [11].

Statistical analysis. Data were analyzed using Epi Info software, version 6.04b (CDC) and LogXact, version 2.0 (Cytel Software). Mantel-Haenszel matched ORs (MORs) were calculated, and 2-tailed P values of $<.05$ were considered to be

statistically significant. LogXact, version 2.0, was used to calculate the MORs when there were no pairs for which the control subject was exposed and the case patient was not.

RESULTS

Epidemiologic and clinical information. A total of 494 SN isolates were reported to the CDC during the period of 1 November 1999 through 31 January 2000. Of these isolates, 248 (50%) received in 21 state laboratories were analyzed by PFGE; 78 (31%) had patterns that were indistinguishable, which was identified as the outbreak pattern (PulseNet number JJPX01.005) (figure 1). The dates of illness onset were known for 53 persons infected with the outbreak strain, and they fell in the period of 13 November to 27 December 1999 (figure 2). Among the 72 patients for whom complete data were available, the median age was 37 years (range, 3 months to 91 years), 44 (61%) were female, and 30 (42%) were Asian, Latino, or Arab. There were 15 hospitalizations and 2 deaths (table 1). One of the patients who died was a 79-year-old, apparently healthy woman, and the other was a 60-year-old man with multiple myeloma.

Case patients resided in 13 states in the United States: California (29 patients), New York (10 patients), Virginia (8 patients), Massachusetts (6 patients), Connecticut (5 patients), Maine (2 patients), Colorado (4 patients), Pennsylvania (4 patients), Georgia (4 patients), Rhode Island (3 patients), Texas (1 patient), Ohio (1 patient), and Minnesota (1 patient). During 1999, of the 136,704 cases of salmonellosis reported in Europe to EnterNet, 686 (0.5%) involved SN, with no significant increase reported during the outbreak period (Ian Fisher, personal communication).

The hypothesis-generating interviews revealed that 65% of people reported eating mango or cilantro 5 days before the onset of illness. Food items included in the case-control study questionnaire were those that were eaten by ⩾50% of those interviewed during hypothesis-generating interviews, as well as foods that were often implicated in previous outbreaks of salmonellosis. A total of 53 case patients were interviewed by telephone. The other 26 case patients were either unreachable after 3 attempts (18 patients), refused to be interviewed (6 patients), or had died (2 patients). Of the 53 patients, control subjects were identified for 28 (53%). Enrolled patients were not significantly different from those excluded with respect to age, sex, or ethnicity. Fourteen case patients were matched with 2 control subjects each, and 14 were matched with 1 control subject each, for a total of 28 case patients and 42 control subjects.

Table 1. Symptoms and characteristics of illness among patients infected with *Salmonella enterica* serotype Newport, January 2000.

Symptom or characteristic of illness	Value
Diarrhea	53/53 (100)
Fever	44/48 (92)
Abdominal cramps	36/51 (71)
Nausea	27/48 (56)
Vomiting	23/51 (45)
Bloody diarrhea	15/50 (30)
Date of illness onset	13 Nov–27 Dec
Duration of diarrhea, median days (range)	5 (1–30)
Physician seen for illness	51/53 (96)
Received antibiotic therapy	39/50 (78)
Hospitalized overnight	15/53 (28)
Duration of hospitalization, median nights (range)	3 (1–7)

NOTE. Data are no. of patients with symptom or characteristic/no. of patients examined (%), unless otherwise indicated.

Among 20 food exposures included in the study, mangoes had been eaten by 14 (50%) case patients and 4 (10%) control subjects in the 5 days before the patients became ill (table 2). In matched analysis, only mango consumption was significantly associated with illness (MOR, 21.6; 95% CI, 3.53–∞; $P = .0001$).

Of the 53 case patients interviewed (including those excluded from the matched case-control study), 27 (51%) recalled eating mangoes in the 5 days before illness onset. Of these, all ate the mangoes uncooked, 18 (67%) washed the mango before eating, and 6 (22%) bit into the mango with the peel intact. Among the 4 control subjects interviewed who ate mangoes, 3 ate the mango uncooked, 1 washed it before eating, and 1 bit into it with the peel intact.

Traceback and environmental investigation. Four case patients in 3 states were able to recall the name and location of a single venue where they purchased the mangoes. Traceback

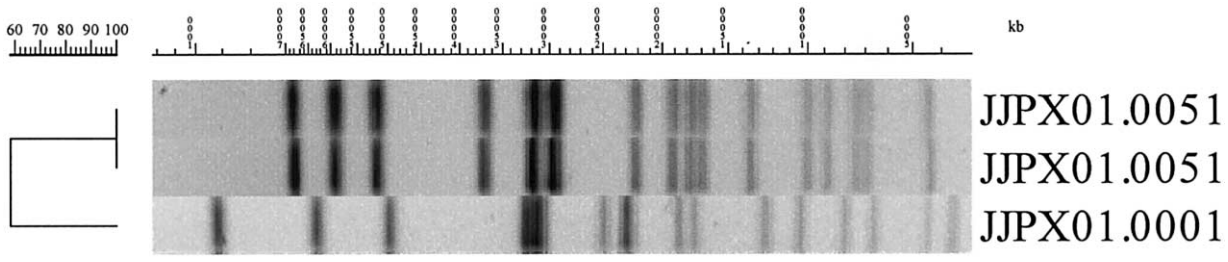

Figure 1. PFGE patterns of the control *Salmonella enterica* serotype Newport isolate (JJX01.0001) and of the outbreak strains (JJPX01.0051)

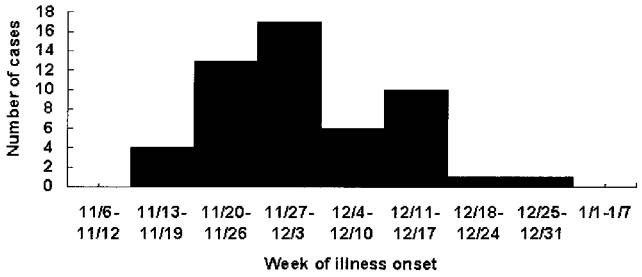

Figure 2. Time (month/week) of illness onset among 53 patients in the United States infected with *Salmonella enterica* serotype Newport with the outbreak PFGE pattern, 6 November 1999 through 1 January 2000.

of the mangoes purchased and consumed by these 4 patients showed that, although there was no common store, distributor, importer, or shipment, a single farm, "farm A" in Brazil, supplied mangoes to all 4 venues.

In June 2000, FDA, USDA/APHIS, and CDC representatives visited farm A. The farm was not in operation at this time because harvest season had ended. All mango farms that export to the United States, including farm A, are under the supervision of APHIS, which is responsible for preventing importation of plant pests, such as the Mediterranean fruit fly. Farm A processes ~11 tons of mangoes from October through December; ~60% of these mangoes are exported to the United States, ~30% are exported to Europe, and ~5% are exported to Argentina; ~5% are consumed in Brazil.

Mangoes are harvested by hand and transported to an onsite processing plant; those that have fallen from the tree are not collected for export. Mangoes, at ambient temperature, are first washed in water that is also at ambient temperature and is chlorinated (to an estimated concentration of 100 mg/L) once per day and not routinely changed unless it becomes grossly turbid. The chlorine level is not measured or monitored. Mangoes are taken in a single layer by conveyer belt for stem removal and detergent wash (pH, 11–13) and pass under a series of brushes for maximum of 20 min to remove soil, pesticide residues, and insects clinging to the surface. The mangoes are then fan dried and sorted for different export markets.

Mangoes destined for the United States undergo a hot water treatment to eliminate fruit fly infestation, followed immediately by a cool water treatment to cool the fruit before packaging [12]; mangoes destined for European and South American markets are packaged without additional hot water treatment. For the disinfestation, crates of mangoes are dipped in hot water (temperature, 46.7°C) for 75–90 min, depending on mango size [12]. Although it is not mandatory, at farm A, treated mangoes are immediately placed in a cool water tank (temperature, 21.1°C) for 6–10 min, which maintains a steady temperature using a hydrocooling system. The hot dip water is not chlorinated; the cool dip water is chlorinated (concentration, ~100 mg/L) once per week, but chlorine levels are not monitored. Water is changed at least once per week; the water is changed more frequently if it is turbid. All dip tanks are unenclosed, and toads, birds, and droppings of bird feces were noted near or in the tanks. After the cool water dip, mangoes are waxed using wax mixed with chlorinated water (chlorine concentration, ~4 mg/L). Finally, mangoes are cooled to 12.8°C by air coolers to retard ripening and are packed in cardboard boxes.

The water for processing mangoes at farm A comes via open canals from a river 26 km away. When it arrived at farm A, the water was turbid and was used, unfiltered and unchlorinated, for pesticide and fungicide application and for drip irrigation of mango fields. Some of the water is pumped through a pressure sand filter into a 70,000-L storage tank, which is chlorinated daily (concentration, ~4 mg/L), although chlorine levels are not monitored. This water is used for postharvest processing of mangoes.

Laboratory results. The outbreak PFGE pattern observed for 78 of 248 SN isolates tested was not previously observed in the PFGE database at the CDC, and it was not seen in any of the 15 SN isolates collected after 27 December 2000. The 18 isolates tested were all susceptible to the panel of antimicrobial agents.

One water sample obtained from the open canal upstream from the sand filter yielded an MPN of *E. coli* of 29 organisms per 100 mL; a second sample obtained immediately downstream from the sand filter but upstream from the 70,000-L storage tank yielded an MPN of *E. coli* of 39 organisms per 100 mL. Both water samples yielded *Salmonella* group G (SN is group C2). One of 2 toad cloacal samples yielded *Salmonella* serotype Muenchen and *Salmonella* 4,12,27:-:1,6 (potassium cyanide positive, subspecies I).

After the current investigation, a simulation of the disinfestation process used at farm A showed that 80% of green mangoes internalized *Salmonella* when they were first dipped in hot water

Table 2. Frequency of exposure to selected foods among case patients and control subjects in an outbreak of salmonellosis in multiple states, January 2000.

Food exposure	No. (%) of persons exposed: Patients (n = 28)	Control subjects (n = 42)	Matched OR (95% CI)	P
Mango	14 (50)	4 (10)	21.6 (3.53–∞)[a]	.0001
Tomato	14 (50)	21 (50)	0.95 (0.30–3.16)	.57
Alfalfa	2 (7)	2 (5)	1.60 (0.12–23.84)	.48
Raw egg	5 (18)	2 (5)	7.00 (0.60– 315.00)	.08
Ground beef	11 (39)	24 (57)	0.43 (0.11–1.44)	.10
Shrimp	10 (36)	11 (26)	2.33 (0.53–15.6)	.16
Chicken	22 (79)	32 (76)	1.17 (0.13–14.16)	.62

[a] LogXact, version 2.0 (Cytel Software), was used to calculate the matched OR.

and then cool water containing fluorescent-labeled *Salmonella* [13].

DISCUSSION

We describe a large multistate outbreak of salmonellosis caused by eating imported mangoes. The ethnic distribution of the affected patients resembles that of mango eaters, who are more likely to be Latino or Asian than is the general American population [14]. A single farm, farm A, was identified by traceback as the most likely source of the mangoes. We identified the hot and cool water treatments as a plausible mechanism of contamination on the farm.

The detection of *E. coli* and *Salmonella* species in the source water and in a toad with access to the processing tanks indicates that mangoes on this farm are processed using contaminated water. This water may have become contaminated for several reasons, including exposure to animals during transport through canals, and dip tanks were open to contamination during processing. Strikingly, no outbreak of SN infection was detected in Europe, which received otherwise identical mangoes imported from farm A that were not dipped in water for fruit fly control. This suggests that the disinfestation treatments are the most likely source of contamination of mangoes.

Normally, fruit skin acts as a physical barrier to pathogens. However, when warm fruit is placed in cooler water, gases within the fruit contract during cooling, and an inward hydrostatic force can draw in water—as well as pathogens [15–17]. One study demonstrated internalization of *Salmonella* species through the stem scar of tomatoes when the temperature of the water was 15°C cooler than that of the tomato [18]. Similar internalization of bacteria has been demonstrated in apples [16], oranges [17], and, most recently, mangoes [13].

Since 1988, the USDA/APHIS-mandated hot water treatment for Mediterranean fruit fly control has been required for all importers of mangoes, as well as growers in Florida who ship mangoes to California. South American mango exporters began using the USDA/APHIS-mandated hot water dip process in the early 1990s (P. C. Witherell, personal communication). The hot water dip replaces ethylene dibromide fumigation, which is being eliminated because of carcinogenicity [19]. The microbiological impact of the heat treatment was not considered before its widespread implementation.

After this investigation, USDA/APHIS recommended that all mango producers who export to the United States ensure that processing water is filtered and adequately chlorinated, with measurement of chlorine levels, and it required that, if cool water dips are used, a minimum of 30 min should elapse between hot and cool water dips [20]. Irradiation is an alternative treatment for Mediterranean fruit fly control. FDA approved low-dose irradiation as a quarantine treatment for fruit and vegetables in 1986; since then, USDA/APHIS has proposed regulations supporting irradiation treatment to disinfest fruits such as mangoes and papayas [21].

Traditionally, outbreaks are detected when a localized group of individuals consume a common food item or their exposure is in a limited geographic area—the classic "church supper" scenario. However, improved transportation and food preservation technologies have resulted in wider geographic distribution of food products. Even fresh produce, with its propensity for spoilage, can be transported efficiently across the world. To keep pace with this changing foodborne outbreak scenario, new public health tools, such as PulseNet, have been developed. In the present example, an outbreak was initially suspected when 5 SN isolates recovered from patients who were ill during the same month had indistinguishable PFGE patterns. Analysis of SN isolates by PFGE provided increased precision in defining cases included in the epidemiologic study. The single PFGE pattern suggested there was a common point of contamination.

The traceback investigation involving 4 independent case patients identified farm A as the only common link. This link would be stronger if we had identified SN on the farm, although the farm investigation took place many months after the outbreak had occurred. In 1999, the United States imported ~20,628 metric tons (MT) of mangoes; 10,376 MT (50%) were from Brazil [22], of which only 6.6 MT (0.06%) were from farm A, making it less likely that we identified farm A solely because it was a major mango supplier to the United States during the outbreak period.

It is likely that the magnitude of this outbreak involved more than the 78 cases identified. Not all SN isolates were subtyped by PFGE. An estimated 38 *Salmonella* infections occur for every 1 case reported through laboratory-based *Salmonella* surveillance [23]; therefore, 3000 cases of SN infection may have occurred during November and December 1999. The underreporting is a reflection of the inherent limitations of passive surveillance. It is critical that clinicians obtain stool specimens from patients who have diarrhea and fever, who have bloody diarrhea, or who may be part of an outbreak [24]. Without this vigilance, there is no possibility of detecting and subsequently preventing foodborne outbreaks.

In summary, new technologies allow the shipping of produce across borders without also shipping agricultural pests, such as Mediterranean fruit flies. Unfortunately, these same technologies may create opportunities for produce contamination with human pathogens unless specific safeguards are present to prevent them. This outbreak demonstrates the need to evaluate the microbiologic impact of new food processes as they are developed or implemented, even when the intended target of the process is not microbial. To this end, collaboration among epidemiologists, food microbiologists, and the international

produce trade community to investigate outbreaks and design control measures is critical to protecting our food supply.

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