

A Multistate Outbreak of *Salmonella enterica* Serotype Typhimurium Infection Linked to Raw Milk Consumption—Ohio, 2003

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ABSTRACT

In December 2002, the Ohio Department of Health was notified of two children with *Salmonella* infection. Both had a history of drinking raw milk from a combination dairy-restaurant-petting zoo (dairy). The dairy was the only establishment in Ohio licensed to sell raw milk and reported 1.35 million visitors annually. We investigated to determine the extent of the outbreak and identify illness risk factors. A case patient was any person with pulsed-field gel electrophoresis–matched *Salmonella enterica* serotype Typhimurium from 30 November 2002 to 18 February 2003. Sixty-two met the confirmed case definition. Forty dairy case patient patrons were included in a case-control study; 56 controls were their well meal companions. Consumption of raw milk was found to be associated with illness (odds ratio, 45.1; 95% confidence interval, 8.8 to 311.9). The dairy discontinued selling raw milk. Because 27 other states still allow the sale of raw milk, awareness of the hazards of its consumption should be raised and relevant regulations carefully reviewed.

Each year in the United States, foodborne disease causes approximately 76 million illnesses. Of these, an estimated 1.4 million (1.8%) are due to *Salmonella* infection, resulting in more than 16,000 hospitalizations and 580 deaths (24). Furthermore, recent work has shown excess mortality associated not only with acute infection but also up to 1 year after infection (20).

Salmonella infection can be contracted by eating raw or undercooked eggs and egg products, raw milk, contaminated water, meat, poultry, and foods cross-contaminated from these sources. Pet turtles, lizards, and chicks are also potential sources of infection (12). Raw milk consumption has also been associated with several other infectious diseases (12, 17).

In Ohio, salmonellosis is a reportable disease. Laboratories that identify *Salmonella* are required to report it to the local health department (LHD) within whose jurisdiction the patient resides (2). *Salmonella* isolates are sent to the Ohio Department of Health (ODH) laboratory for serotyping and pulsed-field gel electrophoresis (PFGE). The LHDs report case patients to the ODH.

On 3 December 2002, the infection-control practitioner at a local hospital reported two children infected with *Sal-*

monella to the LHD. After a preliminary investigation, on 10 December 2002, the LHD notified the ODH. On 13 December 2002, the ODH laboratory identified the serotype as *Salmonella enterica* serotype Typhimurium. The initial investigation by the LHD suggested that the source for the *Salmonella* Typhimurium infection was raw, unpasteurized milk purchased at a local combination dairy-restaurant-petting zoo (dairy) between 27 November 2002, the day the first case patient visited the dairy, and 13 December 2002, the day the dairy stopped selling raw milk following an order from local health authorities.

The dairy in this outbreak was a family-operated business established in 1958. It consisted of a working dairy farm, restaurant, snack bar, and a petting zoo with goats, cows, lambs, and pigs. At the time of the epidemiologic investigation in December 2002, 211 employees, including 16 members of the owner family, worked at the dairy. In 2002, intrastate sale of raw milk for human consumption was legal in 28 states, including Ohio (2002 population: 11 million) (17). As of October 1997, Ohio law did not allow the sale of raw milk except by dairies continuously in the business of selling or offering it for sale directly to consumers before 31 October 1965 (3). Since 1958, the dairy has sold raw milk to the public in jugs. In the 1960s, raw milk sales by the glass and in milkshakes started. In December 2002, the dairy was the only Ohio establishment that legally sold raw milk in jugs and served raw milk and milkshakes to customers. For 2001, the dairy reported ap-

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proximately 1.35 million customers. We investigated to determine the extent of the outbreak and to identify risk factors for illness. In this paper, we report on the findings of this outbreak investigation.

MATERIALS AND METHODS

Case ascertainment. We defined a suspected case patient as any person with either at least one symptom of gastrointestinal tract infection (diarrhea, cramps, fever, chills, nausea, or vomiting) or *Salmonella* spp. isolated from a clinical sample collected from 30 November 2002 to 18 February 2003 and who (i) was a dairy customer from 27 November 2002 to 18 February 2003 (primary infection); (ii) was a friend or household contact of the primary infected case patient (secondary infection); or (iii) worked at the dairy during the outbreak. A confirmed case patient was a suspected case patient with a PFGE-matched *Salmonella* Typhimurium isolate.

Case finding was conducted by reviewing laboratory culture results from hospital, private, and ODH laboratories, comparing PFGE patterns of *Salmonella* Typhimurium isolates received at the ODH laboratory, and posting the pattern on the Centers for Disease Control and Prevention (CDC) PulseNet Webboard for comparison among isolates at the participating PulseNet laboratories (9), alerting public health officials of the outbreak, interviewing meal companions of case patients, and screening dairy workers. Using the CDC's Epidemic Information Exchange (Epi-X) Web site, other state health departments were requested to report cases of *Salmonella* Typhimurium linked to the dairy. Media coverage promoted public awareness of the outbreak.

Case-control study. To verify the initial findings that implicated raw milk and to identify other potential sources of infection, we conducted a case-control study. Confirmed case patients who ate at the dairy were included in the study. Controls were a convenience sample of well meal companions of case patients. Because of numerous potential exposures to *Salmonella* Typhimurium, dairy workers were excluded from the study; secondary infections among friends or household contacts of case patients were also excluded.

Case patients and controls (or their parents if the case patient or control was a child) were interviewed by telephone using a structured questionnaire. We collected information on clinical and food history and animal exposures at the dairy. The questionnaire contained a list of food items offered in the restaurant and the snack bar, as well as a list of petting zoo animals.

Since the dairy stopped selling raw milk in all forms (skim, whole, and cream) on 13 December 2002, the exposure to raw milk and raw milk products was defined as having milk, milkshakes, or coffee with fresh milk at the restaurant during 27 November to 13 December 2002 or purchasing raw milk that was bottled before 13 December 2002 and drinking it later. The analysis of food items served only in the restaurant was restricted to case patients and controls who had a meal at the dairy during 27 November 2002 to 18 February 2003 (the last day the ODH laboratory reported test results of cases potentially related to the outbreak).

Statistical analysis. A database was created, and univariate odds ratios (ORs) with corresponding 95% confidence intervals (CIs) associating examined risk factors with illness were calculated with Epi-Info software, version 6 (14).

Laboratory investigation. Stool specimens from patients with suspected *Salmonella* infection were cultured with MacConkey and Hektoen Enteric agar (BD Diagnostic Systems,

Sparks, Md.). Second-day MacConkey and Hektoen Enteric plates were used after part of the specimen was grown in selenite broth enrichment (BD Diagnostic Systems). Suspected colonies were inoculated onto a triple sugar iron and tryptic soy agar slants (BD Diagnostic Systems), and typical-looking *Salmonella* reactions (16) were confirmed by using an API 20E rapid test strip (bioMérieux, Durham, N.C.). Samples of dairy products and animal and environmental specimens were similarly cultured and all enteric plates reviewed for typical colony morphology.

Isolates were screened for the O antigen using the tryptic soy agar slant and tested against pooled H antisera using H broth (5). Serotyping was completed by testing against individual antisera. The *Salmonella* Typhimurium isolates were subtyped by PFGE (BIO-RAD Mapper System or BIO-RAD GenePath System, Bio-Rad Laboratories, Hercules, Calif.) and treated with restriction endonuclease *Xba*I (New England Biolabs, Beverly, Mass.). The image of the PFGE pattern (BIO-RAD GelDoc System) of the outbreak-associated isolates was compared with other Ohio *Salmonella* Typhimurium isolates (GelCompar, Applied Math, Austin, Tex.) and submitted to the CDC PulseNet Webboard. Additionally, isolates were sent to the CDC for phage typing (6) and antimicrobial susceptibility testing (Sensititre, Trek Diagnostics, Westlake, Ohio).

Worker screening. The LHD required stool samples for *Salmonella* testing from all workers at the dairy. Employees submitted a stool sample using the Cary and Blair transport medium (11). Samples were tested at the ODH laboratory. Clinical history was obtained from employees who tested positive for *Salmonella* Typhimurium.

Environmental investigation. Local and state investigators reviewed milking, bottling, and capping procedures and inspected the dairy. Samples from the dairy environment, food offered at the restaurant, and dairy cows' manure were collected for testing.

RESULTS

During 30 November 2002 to 18 February 2003, the ODH laboratory received 94 *Salmonella* Typhimurium isolates for PFGE testing, including one from Indiana. Two isolates from suspected cases were not available for testing. Fifty-nine isolates from Ohio and the one from Indiana had an indistinguishable pattern and were considered to be associated with the outbreak (Fig. 1). The patterns of two additional isolates from Tennessee and Illinois matched the Ohio pattern. On 18 February 2003, the ODH laboratory reported the last PFGE pattern related to the outbreak.

Patient characteristics. Of 161 interviewed, 43 persons met the suspected case definition, 62 met the confirmed case definition, and 56 were asymptomatic. The 62 confirmed case patients included 40 customers, 6 household contacts, and 16 (7.6%) of 211 dairy workers. The 40 customers were residents of three states (Ohio, Illinois, and Indiana). Their median age was 8 years (age range, 1 to 69 years); 16 (40.0%) were males. All 40 customers reported symptoms, including diarrhea (39 [97.5%]), cramps (32 [80.0%]), fever (31 [77.5%]), chills (27 [67.5%]), body aches (26 [65.0%]), bloody diarrhea (21 [52.5%]), vomiting (21 [52.5%]), nausea (20 [50.0%]), and headache (18 [45.0%]). The date of visit was known for 27 of the 28 patrons who ate at the restaurant (27 November to 8 December 2002). Of the 22 customers who purchased milk,

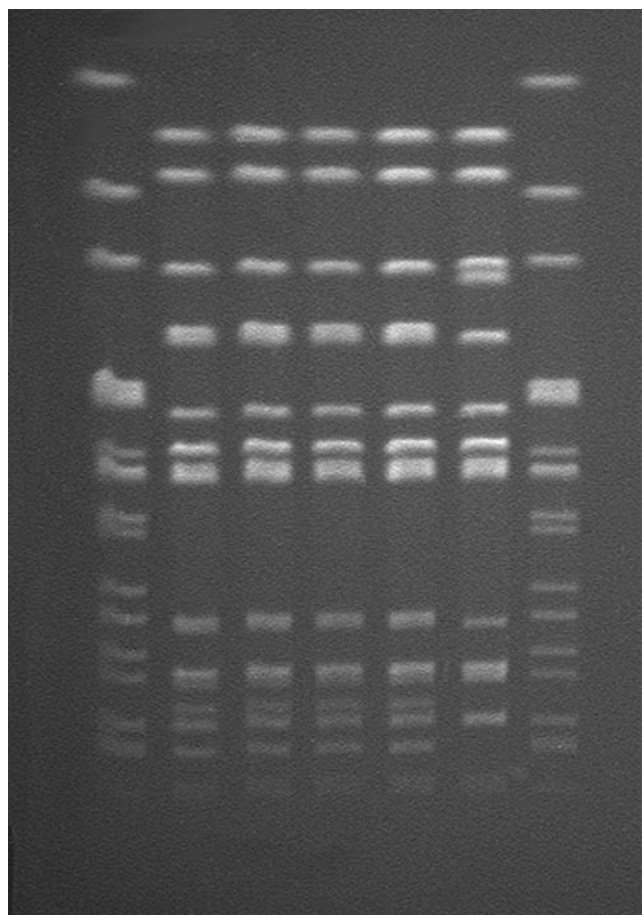


FIGURE 1. Image of pulsed-field gel electrophoresis patterns (BIO-RAD GelDoc System, Bio-Rad, Hercules, Calif.) of selected *Salmonella Typhimurium* isolates restricted with *Xba*I enzyme (New England Biolabs, Beverly, Mass.). Lanes 1 to 7, from left to right. Lanes 1 and 7 show *Salmonella* standard a.m. O1144. Lanes 2 through 5 show the pattern associated with the outbreak. Lane 6 shows the pattern of a strain associated with sporadic infection.

17 recalled the date the milk was used for consumption (28 November to 9 December 2002). The disease onset dates ranged from 30 November to 26 December 2002 (Fig. 2).

There were six household contacts identified; all were residents of Ohio and Tennessee (median age, 5.5 years; age range, 1 to 49 years; 2 [33.3%] were males). Their

disease-onset dates ranged from 14 December 2002 to 14 January 2003 (Fig. 2).

Case-control study. Forty case patients, all customers of the dairy, and 56 controls were eligible for the case-control study. The controls ranged in age from 1 to 74 years (median age, 35 years); 22 (39.3%) were males. In the univariate analysis of potential risk factors, only consumption of raw milk was found to be significantly associated with illness. Among case patients and controls with known dates of milk purchase from the dairy, case patients were significantly more likely than controls (37 [94.9%] of 39 versus 16 [29.1%] of 55) to have consumed raw milk (OR, 45.1; 95% CI, 8.8 to 311.9). None of the other food items analyzed (omelets, egg dish, ice cream, pancakes, pork dish, ham dish, chicken dish, turkey dish, beef dish) or visiting the petting zoo and petting animals were associated with illness.

Workers. On 14 December 2002, the LHD distributed stool kits to workers. Of the 211 workers, 199 (94.3%) provided stool samples for testing. Of these, 16 (8.0%) were culture positive for *Salmonella Typhimurium*, including six restaurant workers, four barn workers, four snack bar workers, and two management and administration workers. The 16 workers ranged in age from 18 to 70 years (median age, 27 years); 10 (62.5%) were males. Eight workers (50.0%) reported symptoms, including diarrhea (7 [43.8%]), cramps (5 [31.3%]), fever (4 [25.0%]), chills (1 [6.3%]), body aches (1 [6.3%]), bloody diarrhea (5 [31.3%]), vomiting (2 [12.5%]), nausea (4 [25.0%]), and headache (1 [6.3%]). Seven (43.7%) reported a disease onset from 4 December to 21 December 2002 (Fig. 2).

Dairy operation. The dairy operation had been issued two licenses, one for retail sale of raw milk and one for manufacturing frozen desserts. Five workers were involved in processing milk. Four workers operated the barn and milked cows, and the fifth employee bottled milk. The cows were mechanically milked twice a day using a milking machine and claws. The animals' teats were treated with a sanitizing solution before the time of milking. Milk was collected into buckets and manually poured into the milk transfer station. At this point, bigger debris, such as straw or grass, was caught on the strainer pad. Milk from the

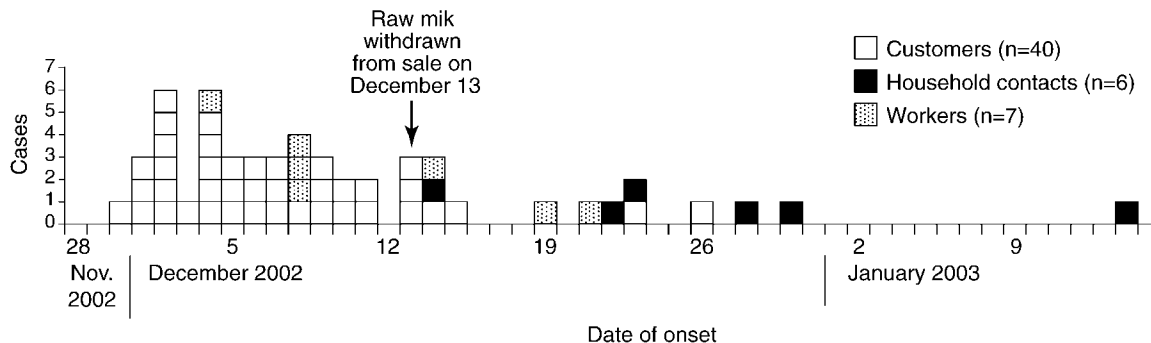


FIGURE 2. Number of patients with symptomatic *Salmonella Typhimurium* infections, by illness onset, 30 November 2002 to 14 January 2003 (onset date not known for one case patient; n = 53).

station was transferred to and stored in a bulk tank. When enough milk was collected, it was bottled in 0.5- and 1-gal (1.89 and 3.79 liters, respectively), disposable jugs. As required by Ohio law, milk jugs were labeled with the words, "This product has not been pasteurized and may contain disease-producing organisms." No program was established for evaluating raw milk quality for retail.

In the dairy operation, one person bottled and hand-capped milk jugs. The same person made all the ice cream at the facility. Milk bottling and ice cream making were done in the same room. The results of worker screening tests revealed that four barn workers had asymptomatic *Salmonella* Typhimurium infection. The site investigation also revealed that in the snack bar, no warning informed customers that raw milk was used in the milkshakes. The warning was present on the menu in the restaurant.

Environmental samples. On 26 December 2002, the Ohio Department of Agriculture (ODA) Dairy Division collected and tested 17 environmental samples, and the owner of the dairy provided results of six additional environmental samples from equipment and working areas. All test results were reported as negative.

Food samples. The ODH laboratory tested 32 food samples collected from 10 December 2002 to 8 January 2003, including ice cream (19 samples), skim raw milk (four samples), whole raw milk (three samples), butter (two samples), whipping cream (two samples), cream (one sample), and buttermilk (one sample). Raw skim milk samples were taken from different 1-gal jugs. The buttermilk sample was collected from the buttermilk made by a patient who mixed raw milk with commercial buttermilk. The butter samples were taken from butter made from raw milk by customers in their homes.

Of the 32 samples, five tested positive for *Salmonella* Typhimurium, including three skim milk samples, one sample of butter, and one sample of cream. All skim raw milk samples were taken from the milk either bought or bottled on 29 November. The butter and cream were both made from skim raw milk.

Animal samples. On 23 December 2002, stool samples from 31 dairy cows that provided milk were collected and submitted to the ODA laboratory for testing. None of them tested positive for *Salmonella*.

Subtyping and phage typing. PFGE analysis has been completed on all available human ($n = 62$) and food ($n = 5$) *Salmonella* Typhimurium isolates associated with the outbreak. All patterns were indistinguishable and are referred to as PulseNet pattern JPXX01.03029 (Fig. 1). Two phage-typed isolates were definitive type 170 and were susceptible to tested antibacterial agents.

DISCUSSION

We conclude that this large, multistate outbreak of *Salmonella* Typhimurium was caused by consumption of raw milk obtained at the dairy. Although animal and environmental samples were negative for *Salmonella* Typhimurium, investigators found that four milk processors were

infected with *Salmonella* Typhimurium. Additionally, all *Salmonella* Typhimurium isolates from clinical specimens and foods had indistinguishable PFGE patterns. The mechanism of contamination was not determined, although the findings suggest contamination of milk may have occurred during the milking, bottling, or capping process.

In 1987, the U.S. Food and Drug Administration banned interstate shipping and sale of raw milk (17). In addition, some states have banned intrastate sale. Ohio law does not allow the sale of raw milk except by dairies in operation before 1965. The dairy in this outbreak was the only place in Ohio legally selling raw milk. In response to the ODA recommendations on 16 January 2003 that the dairy no longer sell raw milk, the dairy owner voluntarily relinquished the dairy's license to do so.

Numerous outbreaks have been investigated in which raw milk was the vehicle for transmission of pathogens, clearly indicating that raw milk poses a risk for human health (17). During 1972 to 2000, a total of 58 raw milk-associated outbreaks were reported to the CDC, of which 17 (29%) were caused by *Salmonella* spp. (17, 19). Also, raw bulk tank milk can contain one or more species of pathogenic bacteria, including *Salmonella* spp. (21, 22). Additionally, several newly described pathogens have been associated with raw milk and other foods (7, 13, 26, 28).

The most effective and best method to ensure microbial safety of food is heat treatment. Of the different applications, pasteurization has a great impact in reducing the incidence of milk-borne diseases (7, 29). The process has proven effective in eliminating pathogenic organisms from milk and preventing diseases since 1906 (25, 29). Moreover, it causes only minor changes in the nutritional value of milk. Human nutrition studies have not demonstrated any advantage of raw over pasteurized milk (7, 25). Despite these studies, certain consumers believe raw milk is of better quality and taste than pasteurized milk (18, 19, 23). These consumers argue for their right to freedom of choice of access to raw milk (25). In several states, milk producers circumvent existing regulations and provide raw milk to consumers by establishing cow-leasing programs in which farmers keep and milk cows owned by individuals (8). In other states, milk producers continue to endorse policies that allow marketing and sale of raw milk to the public directly from the farm (27). The ongoing demand for raw milk and continuing occurrence of outbreaks underscore the importance of public education in reducing the potential hazards of raw milk. Rules that govern raw milk retail should be reviewed and strengthened, because they may not be sufficient to protect public health.

As previous investigators highlighted, molecular subtyping of *Salmonella* Typhimurium strains was supportive in investigating and defining the extent of this outbreak (4). Typhimurium is one of the most common serotypes of *Salmonella* isolated from humans in Ohio. Of the 1,602 *Salmonella* isolates reported in Ohio in 2000, 891 were available for serotyping. *Salmonella* Typhimurium was identified in 13.2% ($n = 211$) of all isolates (1). The specificity of PFGE typing helped identify cases that were part of this outbreak.

We faced several limitations in this study. The control group was a convenience sample of well meal companions of the case patients. Because case patients and control group members could have similar preferences and habits, other risk factors for *Salmonella* infection might not have been identified (15). In addition, because we did not test stool samples of control group members, we were not able to exclude persons with asymptomatic infection, which could have resulted in misclassification. This would have likely biased results toward the null. Also, although 43 suspected case patients expressed symptoms of *Salmonella* infection, we excluded them from the study because either stool samples were not available for testing ($n = 41$) or isolates were not available for subtyping ($n = 2$). The time delay from when illness began and when we initiated the case-control study may have contributed to recall bias. Because of difficulty of recall, we were not able to use dates of exposure (e.g., eating at the dairy or consuming raw milk) and onset of disease as criteria for classification of cases, leading to potential misclassification of primary and secondary cases. We attempted to minimize the recall bias with regard to food history by providing a complete list of food items on the questionnaire. Finally, since workers were excluded from the case-control study, the additional information they could have provided about potential sources of infection was not available.

This investigation linked consumption of raw milk and products that contained raw milk to infection with *Salmonella* Typhimurium. Raw milk, in all forms, was removed from sale on 13 December when implicated in the investigation. Because the dairy relinquished its license, no place in Ohio legally sells raw milk. However, because 27 states still allow the sale of raw milk and organizations continue their efforts to market the sale of raw milk to the public directly from the farm, consumer education about the hazards of raw milk consumption and a careful review of relevant policies are needed.

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