Two Outbreaks of Multidrug-Resistant Salmonella Serotype Typhimurium DT104 Infections Linked to Raw-Milk Cheese in Northern California

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Salmonella causes an estimated 800,000 to 4 million human infections each year in the United States,1 approximately 25% of which are serotype Typhimurium.2 Recent national surveillance data of antimicrobial resistance patterns show that a new multidrug-resistant strain of Salmonella Typhimurium is emerging.3 In 1990, less than 1% of isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline, a distinct 5-drug resistance pattern, but by 1996, 34% of isolates showed this pattern.

This trend appears to be lagging 5 to 10 years behind a similar trend in the United Kingdom, where Salmonella Typhimurium DT104 is now the second-most prevalent type of Salmonella isolated from humans.4 Studies there have linked infection to consumption of chicken, beef,5 and pork6 and contact with ill farm animals.7 The majority of isolates can be further described by a

Context  Salmonella serotype Typhimurium definitive type 104 (DT104), with resistance to 5 drugs (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline), has emerged as the most common multidrug-resistant Salmonella strain in the United States. However, illnesses resulting from this strain have not been associated definitively with a source in this country.

Objective  To determine the source of 2 outbreaks of Salmonella Typhimurium DT104.

Design  Matched case-control study conducted between March 24 and April 5, 1997 (outbreak 1), enhanced surveillance for new cases dating from February 1, 1997 (outbreak 2), and environmental and laboratory investigations.

Setting and Participants  The case-control study included residents of 2 adjacent counties in northern California infected with the outbreak strain of Salmonella Typhimurium var Copenhagen and age-matched controls. For enhanced surveillance, a case was defined as Salmonella Typhimurium infection in a person exposed to fresh Mexican-style cheese.

Main Outcome Measures  Risk factors for infection and source of implicated food.

Results  Outbreak 1 peaked in February 1997; 31 patients were confirmed by culture as having Salmonella Typhimurium var Copenhagen infection, isolates of which showed indistinguishable pulsed-field gel electrophoresis (PFGE) patterns. The outbreak strain was phage type DT104 with the 5-drug resistance pattern. Sixteen cases and 25 controls were enrolled in the case-control study; 15 of 16 Salmonella Typhimurium var Copenhagen cases compared with 14 of 24 matched controls reported eating unpasteurized Mexican-style cheese, (matched odds ratio, 7.9; 95% confidence interval, 1.1-354.9). Enhanced surveillance uncovered outbreak 2, which peaked in April 1997 and was caused by a non-Copenhagen variant of Salmonella Typhimurium. During outbreak 2, Salmonella Typhimurium was isolated from 79 persons who ate fresh Mexican-style cheese from street vendors and from cheese samples and raw milk. The PFGE pattern of the milk isolate matched 1 of the 3 patterns recovered from patients; all strains were phage type DT104b with the 5-drug resistance pattern.

Conclusion  Raw-milk products pose a risk for multidrug-resistant Salmonella Typhimurium DT104 infections.

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characteristic phage typing pattern known as definitive type 104 (DT104). The DT104 complex of closely related phage types includes DT104, DT104b, and U302.

Multidrug-resistant Salmonella Typhimurium DT104 infections are of concern because although the majority of Salmonella infections cause self-limited gastroenteritis that does not require antibiotics, antibiotics can be lifesaving for patients with invasive infections. We report 2 overlapping outbreaks of multidrug-resistant Salmonella Typhimurium DT104 and DT104b infections. This report is the first to definitively link this pathogen to a food source—raw-milk cheese.

**METHODS**

In March 1997, the California state microbial diseases laboratory in Berkeley noticed an unusual increase in the number of Salmonella Typhimurium var Copenhagen isolates forwarded from 2 adjacent counties in the San Francisco Bay area of California. All the isolates were from persons with Spanish surnames. The laboratory used pulsed-field gel electrophoresis (PFGE) to subtype all Salmonella Typhimurium var Copenhagen isolates from northern California collected after February 1, 1997, and designated the predominant pattern the outbreak strain.

**Case-Control Study**

We conducted a matched case-control study between March 24 and April 5, 1997. We defined a case as Salmonella Typhimurium var Copenhagen infection with the outbreak strain in a northern California resident, having illness onset after February 1, 1997, including diarrhea (≥3 stools every 24 hours). We excluded patients who reported contact with another person with diarrhea during the week before illness onset (possible secondary case). We recruited matched controls by asking each patient (or their parent) for the name of 2 people (friend, relative, neighbor, or coworker) who resided in the same community but in a different household. Cases younger than 20 years were matched with controls within 2 years of their age; those aged 20 years or older were matched with adults of any age. We asked cases to recall exposures to various foods, food establishments, and animals during the week before diarrhea onset; we asked controls to recall the same exposures during the same week. All interviews were conducted by telephone by the same interviewer (S.H.C.), who was not blinded to the case-control status of the interviewee; most interviews were conducted in Spanish.

**Case Finding**

We informed all counties in the state that we had found an association between Salmonella Typhimurium var Copenhagen infection and 2 exposures: fresh Mexican-style cheese and a particular local flea market. Counties were asked to obtain clinical, demographic, and exposure histories for all new group B Salmonella cases and investigate any reports of people with gastroenteritis and either of the 2 exposures.

Case finding uncovered a second outbreak (outbreak 2). Numerous persons with acute gastroenteritis reported recent consumption of fresh Mexican-style cheese, and their stool cultures revealed Salmonella Typhimurium (not var Copenhagen); we therefore developed a new case definition. We defined an outbreak 2 case as Salmonella Typhimurium (not var Copenhagen) infection in a person with illness onset on or after February 1, 1997, who had eaten fresh Mexican-style cheese in the week before illness onset. We defined a probable case as gastroenteritis in a person exposed to fresh Mexican-style cheese who had not submitted a stool for culture.

**Traceback Investigation**

We collected all available leftover Mexican-style cheese from patients infected with the Salmonella Typhimurium var Copenhagen outbreak strain and from patients infected with Salmonella Typhimurium. Between April 7 and 11, 1997, county environmental health inspectors visited all the stores named as sources by patients infected with Salmonella Typhimurium var Copenhagen and collected cheese samples. On April 21, 1997, inspectors from the California Department of Food and Agriculture collected more cheese samples from small markets of the variety described by patients infected with Salmonella Typhimurium var Copenhagen. Inspectors also collected cheese samples from flea market vendors, street vendors, and other individuals named by patients infected with Salmonella Typhimurium.

**Laboratory Investigation**

The state microbial diseases laboratory serotyped all Salmonella isolates from human or food sources, using standard methods. They performed PFGE and antibiotic sensitivity analysis of all Salmonella Typhimurium var Copenhagen isolates received from northern California counties during February and March 1997, and a subset of Salmonella Typhimurium var Copenhagen isolates received from eastern California counties during March and April 1997 (data available from the authors). Three Salmonella Typhimurium var Copenhagen isolates and 8 Salmonella Typhimurium isolates were phage typed at the Centers for Disease Control and Prevention, Atlanta, Ga. The California Veterinary Diagnostic Laboratory, Davis, tested selected cheese samples with a fluorophos-alkaline phosphatase assay, a test that distinguishes pasteurized from unpasteurized or incompletely pasteurized dairy products.

**Statistical Analysis**

We calculated the maximum likelihood estimates of the odds ratios (ORs) and 95% confidence intervals (CIs) with Epi Info, Version 6.

**RESULTS**

In February and March 1997, the state microbial diseases laboratory received 63 Salmonella Typhimurium var Copenhagen isolates, 53 of which were from northern California. Thirty-one of the 53 isolates showed the same PFGE pattern, designated the outbreak strain. The remaining 22 isolates represented 7 other patterns.
Case-Control Study
Of the 31 patients infected with the outbreak strain of *Salmonella* Typhimurium var Copenhagen, 3 could not be reached for an interview, 3 were secondary cases, 5 did not have appropriate matched controls, and 4 were identified only after the study was complete, leaving 16 who were included in the case-control study. We matched each case with 1 or 2 controls, for a total of 25 controls. Two exposures were significantly associated with infection. Fifteen (94%) of 16 cases reported eating fresh Mexican-style cheese in the week before illness onset compared with 14 (58%) of 24 controls (matched OR, 7.9; 95% CI, 1.1-354.9). Eight (53%) of 15 cases had visited or eaten foods from 1 local flea market in the week before illness onset compared with 2 (9%) of 23 controls (matched OR, 9.5; 95% CI, 1.2-433.7). Of the 8 cases who had visited or eaten foods from the flea market, only 3 recalled eating cheese purchased at the market. Of the 9 patients not included in the case-control study, 7 reported eating fresh Mexican-style cheese in the week before illness onset, but only 4 had visited the flea market.

Cases were more likely than controls to have taken antibiotics during the month before illness onset (44% vs 17%); however, the difference was not statistically significant (matched OR, 4.1; 95% CI, 0.7-43.1). Nineteen (68%) of 28 outbreak 1 cases interviewed were treated with antibiotics for their diarrheal illness.

Descriptive Epidemiology
We ultimately documented 2 outbreaks. One week after completion of the case-control study, several emergency department physicians called their local health departments to report patients with acute gastroenteritis who had eaten fresh Mexican-style cheese purchased from street vendors. Local public health laboratories subsequently identified group B *Salmonella* in stool specimens from those patients. We initially thought that these patients were part of the *Salmonella* Typhimurium var Copenhagen outbreak (outbreak 1). However, after completion of serotyping, we recognized a second *Salmonella* Typhimurium (not var Copenhagen) outbreak (outbreak 2).

Demographics and clinical characteristics of patients in both outbreaks are listed in Table 1. We identified a total of 31 culture-confirmed cases in outbreak 1 and 79 culture-confirmed cases and 68 probable cases from 51 households in outbreak 2. The epidemic curves in the Figure show how the 2 outbreaks overlapped in time. Both outbreaks were centered in 2 adjacent San Francisco Bay area counties (Santa Clara and San Mateo) in predominantly Spanish-speaking communities.

Outbreak 1 cases were younger than outbreak 2 cases (median age, 2 vs 9 years), and a greater proportion of outbreak 1 cases had bloody diarrhea (68% vs 29%). In outbreak 1, 4 (14%) of 28 case patients were hospitalized and in outbreak 2, 10 (13%) of 79 case patients were hospitalized.

Traceback Investigation
Most outbreak 1 patients purchased cheese from small Hispanic specialty markets; only 3 reported any contact with street vendors. However, because several weeks had elapsed between cheese consumption and the case-control study, none of the outbreak 1 patients had leftover cheese available for analysis. Most could not recall a brand name and described cheese cut from an...
Salmonella Typhimurium Linked to Raw-Milk Cheese

Table 2. Serotype, PFGE Pattern, and Phage Type by Source of Salmonella Typhimurium Isolate—San Francisco Bay Area, 1997

<table>
<thead>
<tr>
<th>Laboratory Characteristics*</th>
<th>Source of Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFGE Pattern</td>
<td>Phage Type</td>
</tr>
<tr>
<td>Salmonella Typhimurium var Copenhagen</td>
<td>1</td>
</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2a</td>
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<td></td>
<td>2b</td>
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<tr>
<td>Total</td>
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*PFGE indicates pulsed-field gel electrophoresis; ND, analysis not done; and ellipses, data not available.

unlabeled bulk round and sold by the pound. A survey conducted by the California Department of Food and Agriculture included cheese from 16 small Hispanic specialty markets in Santa Clara and San Mateo counties. None of these stores showed greater than 350 mU/L of alkaline phosphatase activity (range, 980 mU/L to > 11 000 mU/L), a threshold indicating incomplete pasteurization of milk.\(^9\) Three of these 4 stores had been named by outbreak 1 patients.

In contrast, 80% of the outbreak 2 patients purchased cheese from street vendors, either exclusively or in combination with other sources; many had leftover cheese available for analysis. Twenty-three samples of leftover cheese collected from 10 different outbreak 2 households yielded Salmonella Typhimurium. Environmental health inspectors located 2 cheese vendors who made cheeses and sold them to outbreak 2 patients.

One cheese vendor and his wife made cheese in their small apartment kitchen; cheese collected there yielded Salmonella Typhimurium. They purchased raw milk and rennet from dairy A, a dairy with approximately 400 cows, located 24 km away. One raw-milk sample from a bulk milk tank at dairy A yielded Salmonella Typhimurium. The other cheese maker purchased raw milk from dairy B, located about 80 km from dairy A. This cheese maker used a Mexican brand of rennet to coagulate the milk and made the cheese in a backyard shack where the only source of water for cleaning equipment was a garden hose. Cheese samples collected from the shack did not yield Salmonella. We were not able to visit the cheese vendor.

### Subtyping and Phage Typing

The serotyping, PFGE subtyping, and phage typing results are summarized in Table 2. All of the Salmonella Typhimurium var Copenhagen isolates were subtyped and, by definition, showed a single PFGE pattern (pattern 1). All 3 isolates that were phage typed were DT104. All but 2 of the 31 isolates showed the characteristic 5-drug resistance pattern. One isolate was chloramphenicol-sensitive and not tested further; another patient submitted 2 stool samples and the second yielded an isolate resistant to trimethoprim in addition to the 5 other antibiotics. For outbreak 2, limited resources at the state microbial diseases laboratory precluded subtyping of all 79 Salmonella Typhimurium isolates. Thus, a subset of 30 isolates was selected for subtyping to achieve a representative sample across health jurisdictions and time of submission. Three PFGE patterns (patterns 2, 2a, and 2b) that differed from each other by a single band were observed (data not shown). Patterns 2 and 2a were isolated from cheese and pattern 2a was isolated from the raw milk from dairy A. The same 30 patient isolates, as well as the raw-milk isolate and 5 cheese isolates, were tested for antibiotic sensitivity. All but 1 had the 5-drug resistance pattern (1 patient isolate was resistant to only ampicillin and sulfonamides). None of the isolates were resistant to trimethoprim or ciprofloxacin. The 3 patient isolates, 4 cheese isolates, and 1 milk isolate that were phage typed were all DT104b.

Of note, the PFGE subtype isolated from a patient did not always correspond to the subtype isolated from the cheese that he/she had eaten. In 1 instance, a different subtype was isolated from each of 3 members of the same household, although all had eaten the same cheese. The raw-milk isolate showed pattern 2a, whereas the isolate from cheese made with that milk showed pattern 2, as did isolates from patients who had eaten the cheese. This suggests that the milk and the cheese were contaminated with a combination of PFGE subtypes.

### Comment

This investigation documented 2 overlapping outbreaks of 110 culture-confirmed cases of multidrug-resistant Salmonella Typhimurium DT104 and DT104b, both associated with consumption of fresh Mexican-style cheese in San Francisco Bay area Hispanic communities. This report is the first in the United States to link the pathogen to a food source—raw cow’s milk.

In outbreak 1, we epidemiologically linked Salmonella Typhimurium var Copenhagen DT104 infection to fresh Mexican-style cheese but were not able to obtain cheese samples to confirm the association. In outbreak 2, we obtained multiple cheese samples from patients infected with Salmonella Typhimurium DT104b and from cheese vendors and confirmed that outbreak 2 was caused by cheese made from contaminated raw cow’s milk. We recovered 1 of the outbreak 2 subtypes from raw cow’s milk, street-vended cheese, and patients and recovered the other 2 subtypes from
street-vended cheese and patients. Two features of outbreak 1 strongly suggest that the epidemiologically linked cheese also was made from contaminated raw milk. Some markets named by outbreak 1 patients sold unlabeled raw-milk cheese, making it plausible that patients who bought cheese at these venues could have purchased contaminated raw-milk cheese. In addition, outbreak 1 occurred within the same period, geographic location, and ethnic community as outbreak 2. Together, the results of these 2 outbreak investigations suggest that the sale of homemade raw-milk cheese via street vendors, flea markets, and small specialty markets may be common in San Francisco Bay area Hispanic communities and that products made from raw cow's milk are an important vehicle for the transmission of multidrug-resistant Salmonella Typhimurium DT104.

Multidrug-resistant Salmonella Typhimurium DT104 infections from raw-milk products probably are not limited to the San Francisco Bay area, and the implications of raw milk as a vehicle for this pathogen in the state of California are substantial. The California dairy industry is the sixth largest in the world, and the volume of commercial Mexican-style cheeses sold annually in the state increased from 6.75 million kg in 1988 to more than 20.7 million kg in 1997 (Richard Tate, MA, California Department of Food and Agriculture, oral communication, 1997). The volume of unlicensed Mexican-style cheeses sold annually is estimated to be even greater (Richard Tate, MA, oral communication, 1997).

Although we do not know how much of the unlicensed cheese is made from raw milk, raw-milk drinkers in California are more likely than nondrinkers to be Hispanic. The emergence of a multidrug-resistant pathogen carried by dairy cattle, the increased size of the dairy industry, and the increased demand for fresh Mexican-style cheese that may be made with raw milk portend increasingly severe outbreaks of multidrug-resistant Salmonella Typhimurium DT104 infections. Two other investigations in 1997 suggest that Salmonella Typhimurium DT104 infections from raw cow's milk may be widespread in the United States. As described elsewhere in this issue of the Journal, during January through May 1997, an outbreak of Salmonella Typhimurium DT104 infections in a largely Hispanic community in Washington State was epidemiologically linked to fresh Mexican-style cheese made from raw cow's milk. Additionally, in rural Vermont, transmission of Salmonella Typhimurium DT104 from ill dairy cows to humans was documented in May 1997.

Raw or incompletely pasteurized milk can cause infection from several pathogens, most commonly from Salmonella and Campylobacter, but also from Escherichia coli O157:H7 and Listeria monocytogenes, among others. Nationwide, the majority of raw-milk-associated outbreaks occur in states like California, where intrastate sale of raw milk is legal. In California, it is illegal for a dairy farmer to give or sell milk (pasteurized or unpasteurized) to a person who will then resell the milk or milk products if that person does not have a license to do so; however, these outbreaks demonstrate that this practice still occurs.

Our investigation had 3 limitations. First, the outbreak 2 case definition reflected our initial assumption that the cases belonged to outbreak 1 and that we were simply measuring the magnitude of outbreak 1. This definition biased our case finding and precluded identification of another vehicle. However, the fact that 2 of the 3 PFGE subtypes recovered from cases were also recovered from cheese suggests that cheese was the major, if not the only, vehicle causing infections in outbreak 2. Second, laboratory resource constraints at the time of the outbreaks prohibited comprehensive subtyping of all isolates. Although this was a missed opportunity for a complete laboratory description, this additional information likely would not have changed our conclusions or the actions that we took. Third, we do not know how many different dairies contributed to these 2 outbreaks or whether the outbreaks originated from 1 or more common dairies.

Although the majority of Salmonella infections do not require antibiotic treatment, clinicians should be aware of this new multidrug-resistant pathogen so severe infections that do warrant antibiotics can be treated appropriately. Chloramphenicol resistance is a simple screening test that is both highly sensitive and specific for the 5-drug resistance pattern characteristic of Salmonella Typhimurium DT104.

We identified raw milk as a source of multidrug-resistant Salmonella Typhimurium DT104 infection in California. This outbreak highlights the importance of enforcement of existing laws regulating sale of raw milk and education of consumers about the risks of unpasteurized milk products. Based on the results of this investigation, the California Department of Health Services, California Department of Food and Agriculture, local counties, and the California Milk Advisory Board launched a statewide campaign to educate Hispanic consumers about the health risks of unpasteurized cheese.

Previous Presentation: These results were reported in part at the 35th annual conference of the Infectious Disease Society of America, September 14, 1997, San Francisco, Calif.

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REFERENCES
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In 1816 I was consulted by a young woman labouring under general symptoms of diseased heart, and in whose case percussion and the application of the hand were of little avail on account of the great degree of fatness. The other method just mentioned [direct auscultation] being rendered inadmissible by the age and sex of the patient, I happened to recollect a simple and well-known fact in acoustics,...the great distinctness with which we hear the scratch of a pin at one end of a piece of wood on applying our ear to the other. Immediately, on this suggestion, I rolled a quire of paper into a kind of cylinder and applied one end of it to the region of the heart and the other to my ear, and was not a little surprised and pleased to find that I could thereby perceive the action of the heart in a manner much more clear and distinct than I had ever been able to do by the immediate application of the ear.

—Rene Laennec (1781-1826)