Investigation of Multidrug-Resistant Salmonella Serotype Typhimurium DT104 Infections Linked to Raw-Milk Cheese in Washington State

Rodrigo G. Villar, MD
Mark D. Macek, DDS, PhD
Shawna Simons, MS
Peggy S. Hayes
Marcia J. Goldoft, MD, MPH
Jay H. Lewis, MS
Lynnell L. Rowan, DVM
Denny Hursh, RN
Marianne Patnode, RN, MS
Paul S. Mead, MD, MPH

Salmonellosis is a leading cause of foodborne illness in the United States, resulting in an estimated 800,000 to 4 million infections each year. Approximately 25% of all salmonellosis cases reported to the Centers for Disease Control and Prevention (CDC) are caused by a single serotype, Typhimurium. Known as definitive type 104 (DT104), Salmonella Typhimurium emerged in the United Kingdom. This phage type was first identified among isolates from humans. This phage type has been associated with resistance to multiple antimicrobial agents, including ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, and occasionally fluoroquinolones. Systematic surveys suggest that multidrug-resistant Salmonella Typhimurium DT104 has recently emerged as a cause of human and animal illness in Europe and North America. In early 1997, health officials in Yakima County, Washington, noted a 5-fold increase in salmonellosis among the county’s Hispanic population.

Objectives To characterize bacterial strains and identify risk factors for infection with Salmonella Typhimurium in Yakima County.

Design Laboratory, case-control, and environmental investigations.

Setting and Participants Patients with culture-confirmed Salmonella Typhimurium infection living in Yakima County and age- and neighborhood-matched control subjects.

Main Outcome Measures Food vehicle implication based on case-control study and outbreak control.

Results Between January 1 and May 5, 1997, 54 culture-confirmed cases of Salmonella Typhimurium were reported. The median age of patients was 4 years and 91% were Hispanic. Patients reported diarrhea (100%), abdominal cramps (93%), fever (93%), bloody stools (72%), and vomiting (53%); 5 patients (9%) were hospitalized. Twenty-two patients and 61 control subjects were enrolled in the case-control study. Seventeen case patients (77%) reported eating unpasteurized Mexican-style soft cheese in the 7 days before onset of illness compared with 17 control subjects (28%) (matched odds ratio, 32.3; 95% confidence interval, 3.0-874.6). All case-patient isolates were phage type 104 (DT104b) (n = 32), and 20 (91%) were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. The cheese produced and eaten by 2 unrelated patients was made with raw milk traced to the same local farm. Milk samples from nearby dairies yielded Salmonella Typhimurium DT104. The incidence of Salmonella Typhimurium infections in Yakima County returned to pre-1992 levels following interventions based on these findings.

Conclusions Multidrug-resistant Salmonella Typhimurium DT104 emerged as a cause of salmonellosis in Yakima County, and Mexican-style soft cheese made with unpasteurized milk is an important vehicle for transmission. We postulate that recent increases in human salmonellosis reflect the emergence of Salmonella Typhimurium DT104 among dairy cows in the region. Continued efforts are needed to discourage consumption of raw milk products, promote healthier alternatives, and study the ecology of multidrug-resistant Salmonella Typhimurium.

See also pp 1805 and 1845 and Patient Page.
resistant DT104 now accounts for approximately 30% of all Salmonella Typhimurium infections in the United States.8,9 Despite this, there have been few reported outbreaks. European studies have implicated salami, sausages, and other meat products as sources of infection4,5; however, little is known about vehicles that transmit DT104 in the United States.

In early 1997, health officials in Yakima County, Washington, noted an increase in salmonellosis cases among members of the county’s Hispanic population. In this article, we present results from the ensuing outbreak investigation that identified multidrug-resistant Salmonella Typhimurium DT104 as the causative agent and linked illness with consumption of unpasteurized, homemade Mexican-style soft cheese (queso fresco) made by unlicensed producers and distributed illegally in Yakima County, Washington.

METHODS
Surveillance and Laboratory Investigation
In Washington State, clinical laboratories are required to submit all Salmonella isolates to the Washington State Department of Health Laboratories for confirmation and serotyping. We reviewed these records to determine incidence trends and identify Yakima County residents with recent Salmonella Typhimurium infections. We extracted information on patients’ symptoms and demographic characteristics from case report forms.

Pulsed-field gel electrophoresis (PFGE) was used to subtype Salmonella Typhimurium isolates from Yakima County residents.10 The PFGE patterns were compared using Bio-Rad’s Molecular Analyst Fingerprinting Plus software (Hercules, Calif) using the Dice coefficient and unweighted pair group method. Isolates were sent to the CDC for phage typing11 and antimicrobial susceptibility testing.12

Case-Control Investigation
To identify risk factors for infection with Salmonella Typhimurium, we conducted a case-control study among Yakima County residents. We defined a case as diarrhea in a Yakima County resident with illness onset on or after January 1, 1997, and a stool culture that yielded Salmonella Typhimurium. We conducted hypothesis-generating interviews with 6 patients who met the case definition asking open-ended questions about diet, travel, animal exposures, and contact with ill persons. These patients were excluded from the case-control analysis. For the study, we included 12 case patients (92%) from 1 town and 10 randomly selected case patients (30%) from another town.

We interviewed the index patient in each household and systematically selected 3 age- and neighborhood-matched control subjects using coin tosses and predetermined guidelines to select direction of travel and household choice. We excluded neighborhood control subjects if anyone living in that household had experienced a diarrheal illness in the preceding 6 months. Because more than 90% of patients had Spanish surnames and many spoke only Spanish, we restricted our study to prior to patients and control subjects who identified Spanish as their preferred language for at least 1 family member. We administered the questionnaire in either Spanish or English. We asked patients about exposures in the 7 days before illness onset and control subjects about exposures in the 7 days preceding the interview.

Statistical Analysis
Data were analyzed and matched odds ratios, Fisher exact P values, and 95% confidence intervals were calculated using Epi Info version 6.04a (Centers for Disease Control and Prevention, Atlanta, Ga) and EXACT software (David O. Martin, MD, MPH, Beth Israel Deaconess Medical Center, Boston, Mass).13 A value of P<.05 and 95% confidence intervals that did not include 1 were considered significant.

Environmental Investigation
Based on results from the case-control study, we collected samples of Mexican-style soft cheeses from case households and attempted to contact persons who made the cheese. We visited cheese makers and cultured their products and production supplies. We asked cheese makers where they obtained milk, and when possible visited the source dairies to interview dairy managers about farming practices and illness among workers and animals. We collected rectal swabs from dairy cows and placed them into a Cary Blair medium (Becton Dickinson Microbiology Systems, Cockeysville, Md) for transport. During the 3 months following the case-control investigation, the Washington State Department of Agriculture collected milk samples from milk tanker trucks serving Yakima County dairies. The milk tested came from the dairy implicated in this investigation and from a convenience sample of area dairies. Milk, cheese, and rennet samples for Salmonella were tested by the Washington State Department of Health Laboratories.

RESULTS
Surveillance Data
The incidence of human Salmonella Typhimurium infections in Yakima County increased from 5.4 cases per 100 000 person-years in 1992 to 29.7 cases per 100 000 person-years in 1996 (FIGURE). Between January 1 and May 5, 1997, 54 culture-confirmed cases of Salmonella Typhimurium were reported among residents of Yakima County, equivalent to an annualized incidence of 64.8 cases per 100 000 person-years. In contrast, the incidence of culture-confirmed salmonellosis for the rest of Washington State remained at 3.5 cases per 100 000 person-years for the period from January 1990 to May 1997 (Washington State, unpublished surveillance data).

Among 54 Yakima County residents infected with Salmonella Typhimurium in 1997, the median age was 4 years (range, 0-53 years) and 27 were male (46.6%). Symptoms associated with infection included diarrhea (100%) lasting a median of 7.7 days, fever (93%) with a mean maximal temperature of 39.5°C, abdominal cramps (93%),...
bloody stools (72%), and vomiting (53%). Overall, 5 infected persons (9%) were hospitalized and 3 received intravenous therapy (8%). There were no deaths. The majority (81%) of infected persons lived in Yakima County’s 2 largest cities: 28 in Yakima (48%) (annual incidence, 46 cases per 100 000 person-years) and 19 in Sunnyside (33%) (annual incidence, 162 cases per 100 000 person-years).

Laboratory Investigation

Salmonella Typhimurium isolates from 53 of 54 patients were available for PFGE; 42 were available for phage typing. The PFGE process yielded 3 distinct patterns: 38 were pattern A (72%), 12 were pattern B (22%), and 3 were pattern C (6%). These patterns differed from each other by 3 or more bands. Of 42 isolates phage typed at the CDC, 16 were DT104 (38%), 17 were DT104b (41%), 5 were similar to DT104 (minor variation in typical DT104 phage lysis pattern) (12%), 3 were DT120 (7%), and 1 was DT160 (2%). One isolate did not conform to the phage typing scheme. Among isolates with PFGE pattern A, 8 were DT104 (25%), 17 were DT104b (53%), 4 were similar to DT104 (9%), 2 were DT120 (5%), and 1 was DT160 (3%). All pattern B isolates that were phage typed were DT104; none of the pattern C isolates were phage typed. The surface variant Copenhagen was found on 5 pattern A isolates (13%) and 3 pattern B isolates (25%).

Antimicrobial sensitivity testing was completed on 28 isolates drawn randomly from cases occurring in each of the first 5 months of 1997. Overall, 25 were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline (ACSSuT), and amoxicillin with clavulanate (89%). All 24 PFGE pattern A isolates and 1 pattern B isolate had this resistance pattern. Three PFGE pattern B isolates were resistant only to streptomycin and sulfamethoxazole. Sixteen DT104b isolates, 8 DT104 isolates (73%), and the isolate that did not conform to phage typing demonstrated the ACSSuT resistance pattern.

Case-Control Investigation

We interviewed 22 patients and 61 age-matched neighborhood control subjects (2.8 control subjects per case). The median age for enrolled patients was 4 years (range, 5 months to 37 years) and 55% were male; the median age for neighborhood control subjects was 4 years (range, 6 months to 49 years) and 44% were male. Patients and control subjects did not differ with respect to socioeconomic status, as measured by the presence of a telephone in the home, automobile ownership, the number of months worked by the primary wage earner, or mean family size (P>.10 for all comparisons).

When comparing patients with neighborhood control subjects, infection with Salmonella Typhimurium was associated with eating raw-milk Mexican-style soft cheese (matched odds ratio, 32.3; 95% confidence interval, 3.0-874.6) (TABLE). Seventeen (77%) of the 22 case-patients who reported eating Mexican-style soft cheese in the 7 days before illness compared with 17 (28%) of 61 neighborhood control subjects. Among 17 case-patients who reported eating Mexican-style soft cheese, 16 (94%) ate only raw-milk cheese (12 obtained it from an unknown street vendor and 4 from a friend or relative), and only 1 also purchased a commercial, raw-milk Mexican-style soft cheese for the first time recently.

Table. Associations of Exposures Among Patients With Salmonella Typhimurium Infection and Matched Controls, Yakima County, Washington, 1997

<table>
<thead>
<tr>
<th>Food Exposure</th>
<th>No./Total (%)</th>
<th>Odds Ratio (95% Confidence Interval)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case-Patients</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Raw-milk Mexican-style soft cheese</td>
<td>17/22 (77)</td>
<td>17/61 (28)</td>
<td>32.3 (3.0-874.6)</td>
</tr>
<tr>
<td>Other cheeses</td>
<td>7/22 (32)</td>
<td>21/59 (36)</td>
<td>0.8 (0.2-2.7)</td>
</tr>
<tr>
<td>Unpasteurized milk</td>
<td>1/21 (5)</td>
<td>1/55 (2)</td>
<td>Undefined (0.05-∞)</td>
</tr>
<tr>
<td>Chorizo</td>
<td>2/22 (9)</td>
<td>18/61 (30)</td>
<td>0.3 (0.0-1.3)</td>
</tr>
<tr>
<td>Chicken</td>
<td>13/21 (62)</td>
<td>50/61 (82)</td>
<td>0.4 (0.1-1.4)</td>
</tr>
<tr>
<td>Ground beef</td>
<td>9/22 (41)</td>
<td>34/61 (56)</td>
<td>0.6 (0.2-2.1)</td>
</tr>
<tr>
<td>Other beef</td>
<td>13/22 (59)</td>
<td>33/61 (54)</td>
<td>1.4 (0.4-4.6)</td>
</tr>
<tr>
<td>Raw eggs</td>
<td>4/21 (19)</td>
<td>11/58 (19)</td>
<td>1.0 (0.2-4.1)</td>
</tr>
</tbody>
</table>

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pasteurized brand of the cheese from a supermarket. Among the 17 neighborhood control subjects who ate the cheese, 10 (62%) ate a raw-milk product and 7 (44%) ate a commercial pasteurized brand of cheese. Consumption of other dairy products or meats, exposure to livestock, and contact with persons with diarrhea were not significantly associated with illness.

Salmonella isolates from enrolled cases included 19 that were PFGE pattern A (86%), none that were pattern B, and 3 that were pattern C (14%). All 22 case-patient isolates were phage type DT104 (n = 10) or DT104b (n = 12), and 20 (91%) were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline.

Environmental Investigation
Because the shelf life of raw-milk Mexican-style soft cheese is short (approximately 1 week if refrigerated), we were able to collect only a single sample of the implicated cheese. Additionally, the majority of patients were unable to identify the person who sold them the cheese. Nevertheless, we did identify 2 persons living in different towns who independently made cheese eaten by ill persons. Both of these producers purchased unpasteurized milk from the same dairy, 1 of 78 dairies in Yakima County. One cheese maker reported making the cheese for the first time for her family, all of whom became ill after eating it. The second producer regularly made cheese and sold it door to door. The cheese made by the second producer was associated with illness in at least 2 different households. This producer sold cheese primarily in Yakima, but occasionally sold to persons from other parts of the region, including Seattle, Oregon, and California.

We reviewed the cheese making practices of 3 soft cheese producers, including the 2 who had produced cheese consumed by ill persons. Each producer obtained 45 to 60 gallons of unpasteurized milk 2 to 3 times weekly and transported and stored it without refrigeration. The cheese makers allowed the milk to sit overnight at room temperature before adding rennet tablets or liquid to coagulate it. They allowed the milk to stand an additional 1 to 2 hours to set. Excess water was removed and cheese was placed into circular molds. None of these producers boiled or pasteurized the milk. Fresh cheese was sold during the next 5 days.

We visited the dairy where the 2 producers of the implicated unpasteurized cheese purchased raw milk. The dairy had 175 cows and supplied milk for a local creamery. The dairy farmers bred and raised their cows on-site and did not bring cows on the farm from outside sources. The only other animals on this dairy were dogs and cats. The farmers reported that during the first 5 months of 1997 approximately 1 episode of mastitis per month had occurred in the farm’s cows, which were treated with antibiotics, and that no diarrheal illnesses had occurred among the herd.

Cultures of milk from tanker trucks that collected unpasteurized milk from area dairies, including the implicated dairy, yielded Salmonella Typhimurium DT104 on 2 separate occasions. Laboratory testing revealed that both the isolates from the milk were PFGE pattern A and resistant to ACSSuT. Cultures from 5 samples of the cheese made from unpasteurized milk, 2 samples of rennet, 2 samples of unpasteurized milk from the bulk tank of the implicated dairy, and rectal swabs obtained from 5 (3%) of 175 cows on the implicated dairy farm did not yield Salmonella.

COMMENT
Our findings suggest that multidrug-resistant Salmonella Typhimurium DT104 has emerged as an important cause of human salmonellosis in Yakima County and that cheese made with unpasteurized milk is an important vehicle for transmission of Salmonella Typhimurium DT104. Isolation of DT104 from raw milk collected from local dairies supports earlier work indicating that this pathogen is circulating among local dairy cattle.16 Anecdotal reports from cheese makers, consumers, and health officials suggest that the production and sale of raw-milk Mexican-style soft cheese became more common in Yakima County in the early to mid-1990s. This increase in the popularity of quesefresco and other soft cheeses coincided with, and may have been responsible for, the prolonged increase in the incidence of Salmonella Typhimurium infections since 1992. Because epidemiologic studies and patient isolates from earlier years were unavailable, we cannot conclusively demonstrate the role of DT104 or Mexican-style soft cheese in cases occurring before 1997. Nevertheless, the rapid decrease in Salmonella Typhimurium infections to pre-1992 levels following this investigation suggests that the excess in cases seen in 1997 and between 1992 and 1996 were part of a common process.

Almost 90% of Salmonella Typhimurium isolates tested in this investigation were of the multidrug-resistant DT104 group (DT104, DT104b, and similar to DT104 phage types) of which 72% yielded a single pattern when tested by PFGE. In this particular setting, the PFGE pattern appeared to correlate somewhat more closely with the antimicrobial susceptibility of isolates than did the phage type. Whereas PFGE patterns are determined by the presence of restriction sites in the bacterial chromosome, phage type is a phenotype that may be affected by the expression of both chromosomal and extra-chromosomal genes. It is therefore not surprising to find overlap and discordance between these 2 methods of subtyping. Studies characterizing Salmonella Typhimurium DT104 using PFGE have identified at least 10 different profiles, some of which are more common than others (Timothy Barrett, MD, CDC, unpublished data, 1998). A system for identifying Salmonella Typhimurium has not yet been standardized among different laboratories, but PFGE appears to be a promising method for subtyping (Timothy Barrett, MD, oral communication, 1998).

The outbreak in Yakima County represents 1 of 4 recently reported clusters of multidrug-resistant Salmonella Typhimurium DT104 in the United States. The first recognized human outbreak did
not implicate a single source and occurred among school children in 1996 in Nebraska. These children had drunk chocolate milk that was outdated but had also had contact with a turtle and a kitten. As reported elsewhere in this issue, Cody et al investigated another outbreak that occurred in California at the same time as the Yakima County outbreak that also linked illness to consumption of Mexican-style soft cheese made with unpasteurized milk. Additionally, in May 1997, a cluster of illnesses was reported among a family who lived on a dairy farm in Vermont and drank unpasteurized milk. How DT104 was introduced in the United States remains unclear; however, likely sources include contaminated feed, infected farm animals, and possibly migrating birds. Concern exists that multidrug-resistant Salmonella Typhimurium DT104 might be associated with greater morbidity and mortality than infection with other strains of Salmonella. Wall et al reported high rates of hospitalization and mortality among persons with Salmonella Typhimurium DT104 infection in an outbreak in the United Kingdom in 1993. Although there were no deaths or documented invasive infections in the Yakima County outbreak, 5 of the 54 patients with culture-confirmed infection were hospitalized, and the proportion of patients reporting bloody diarrhea (72%) was substantially higher than might be expected based on previous outbreaks or series of sporadic cases.

The multidrug-resistant characteristic of this strain may also predispose to infection persons who have had recent exposure to an antimicrobial. In an unmatched analysis of persons who ate Mexican-style soft cheese, case-patients were more likely than control subjects to have taken antimicrobials in the 30 days before onset of illness (P = .04; data not shown). Although antimicrobial therapy for uncomplicated salmonellosis is generally not recommended, it can be life-saving in cases of invasive illness. We faced several limitations in this investigation. The selection of cases was based on geographic convenience, and may not be entirely generalizable throughout the region. The time delay from when illnesses began and when we initiated the investigation may have contributed to recall bias. However, it is unlikely given the study design that this bias would have been selective for raw-milk Mexican-style soft cheese. Because the investigation occurred several weeks after the majority of persons had become ill, samples of suspect cheese and milk were generally unavailable, limiting our ability to culture the implicated products. Identification of cheese vendors was also difficult; most people did not know the person who sold them the cheese. In Washington State, it is illegal to sell raw milk other than to creameries or licensed individuals, and persons may have been reluctant to reveal their sources. Similarly, the farmer on the implicated dairy allowed us to sample stools from only a small proportion of cows. Based on the results of this investigation, the Yakima County Health District developed several interventions in conjunction with Washington State University’s Cooperative Extension, the Washington State Department of Agriculture, and other community partners. The programs were designed to alert producers and consumers of the risks associated with eating unpasteurized milk products, primarily Mexican-style soft cheese. A major effort went into the development of a program called the abuela (grandmother) project (launched in October 1997). Health educators instructed several Yakima County grandmothers how to make Mexican-style soft cheese more safely using pasteurized milk, thermometers, and guided instruction. The grandmothers in turn taught these techniques to others in the community. Written materials with safe cheese-making recipes and educational messages emphasizing children’s health were distributed through the community in both Spanish and English. Since May 1997, the incidence of Salmonella Typhimurium DT104 infections in Yakima County decreased dramatically. Between June and December 1997, there were only 14 cases reported from Yakima County, dropping the incidence to pre-1992 levels; there were only 2 cases reported in the first 6 months of 1998 (Figure). Continuing prevention measures and surveillance may help determine how effective local interventions are.

In summary, this investigation linked consumption of homemade, Mexican-style, soft cheese (eg, queso fresco), made with unpasteurized milk, to infection with multidrug-resistant Salmonella Typhimurium DT104. Unpasteurized dairy products are excellent vehicles for transmission of Salmonella and other pathogenic bacteria. Unpasteurized cheeses have been implicated in multiple outbreaks and their consumption poses an important health threat. Consumption of unpasteurized dairy products, including Mexican-style soft cheese, should be discouraged, especially among infants, pregnant women, and immunosuppressed persons. Enhancing surveillance for Salmonella Typhimurium DT104 through selective phage typing of multidrug-resistant Salmonella Typhimurium isolates in humans and farm animals may help define the ecology of this emerging pathogen in the United States.

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REFERENCES
While I was listening eagerly to Freud’s lectures I studied assiduously his technique of exposition with a view of modifying my own after him. I wondered how he succeeded in producing something unexpected and stupendous while his talk moved in simple terms, dispensing with the fireworks of baffling profundity or of glittering paradoxes. I found that he made use of Schopenhauer’s recipe for a good style: “Say extraordinary things by using ordinary words.”

—Hanns Sachs (1881-1947)