

## Multistate Outbreak of *Listeria monocytogenes* Associated with Mexican-Style Cheese Made from Pasteurized Milk among Pregnant, Hispanic Women

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### ABSTRACT

Listeriosis is a severe infection caused by *Listeria monocytogenes*. Since 2004, the Centers for Disease Control and Prevention has requested that listeriosis patients be interviewed using a standardized *Listeria* Initiative (LI) questionnaire. In January 2009, states and the Centers for Disease Control and Prevention began investigating a multistate outbreak of listeriosis among pregnant, Hispanic women. We defined a case as an illness occurring between October 2008 and March 2009 with an *L. monocytogenes* isolate indistinguishable from the outbreak strain by pulsed-field gel electrophoresis. We conducted a multistate case-control study using controls that were selected from *L. monocytogenes* illnesses in non-outbreak-related pregnant, Hispanic women that were reported to the LI during 2004 to 2008. Eight cases in five states were identified. Seven of these were pregnant, Hispanic females aged 21 to 43 years, and one was a 3-year-old Hispanic girl, who was excluded from the study. Seven (100%) cases but only 26 (60%) of 43 controls had consumed Mexican-style cheese in the month before illness (odds ratio, 5.89; 95% confidence interval, 1.07 to  $\infty$ ;  $P = 0.04$ ). Cultures of asadero cheese made from pasteurized milk collected at a manufacturing facility during routine sampling by the Michigan Department of Agriculture on 23 February 2009 yielded the outbreak strain, leading to a recall of cheeses produced in the plant. Recalled product was traced to stores where at least three of the women had purchased cheese. This investigation highlights the usefulness of routine product sampling for identifying contaminated foods, of pulsed-field gel electrophoresis analysis to detect multistate outbreaks, and of the LI for providing timely exposure information for case-control analyses. Recalls of contaminated cheeses likely prevented additional illnesses.

*Listeria monocytogenes* is a major cause of hospitalizations and deaths from foodborne infection (1). Pregnant women and newborns, the elderly, and those with conditions associated with severe immunosuppression such as AIDS, cancer, or organ transplant are at increased risk for severe illness.

*Listeria* infection during pregnancy may result in fetal loss, premature labor, neonatal infection, and neonatal death. An estimated 17% of all reported listeriosis cases in the United States occur in pregnant women (7). Because pregnant women and their newborns are at increased risk for *L. monocytogenes* infection, the Centers for Disease Control and Prevention (CDC) advises pregnant women to avoid consumption of high-risk foods such as unpasteurized dairy products, including soft cheeses (2).

Pregnant women with *Listeria* infection during 2004 to 2007 were over three times more likely to report Hispanic

ethnicity than all mothers giving live birth in the United States in 2005 (7). Mexican-style cheese made from raw (unpasteurized) milk has been implicated in multiple outbreaks of *L. monocytogenes*; in these outbreaks, most cases were observed among pregnant, Hispanic women (8, 9). Mexican-style cheeses made from pasteurized milk are not well established as vehicles for *Listeria* outbreaks.

### MATERIALS AND METHODS

**Data sources.** The *Listeria* Initiative (LI) was launched by CDC in 2004 as a tool to aid investigations of listeriosis clusters by using a standardized extended case form questionnaire to obtain food exposures for all human cases (1). To minimize the effect of recall bias on food consumption histories, patient interviews are conducted as individual cases are reported, rather than after clusters are identified. Clinical, food, and environmental *Listeria* isolates are submitted to PulseNet (the National Molecular Subtyping Network for Foodborne Disease Surveillance) for pulsed-field gel electrophoresis (PFGE) to identify clusters of PFGE-matching isolates. Case information from the LI database is linked with

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PFGE information in the PulseNet database, allowing for identification of epidemiologically related and nonrelated illnesses for case-control analysis.

**Case finding.** In early January 2009, PulseNet identified a multistate cluster of five listeriosis cases. A novel two-enzyme PFGE pattern was identified among pregnant, Hispanic women, all of whom had consumed Mexican-style cheeses between September and November 2008. CDC alerted all U.S. state health departments about the cluster and asked states to continue to rapidly perform PFGE analysis of all *L. monocytogenes* isolates, upload PFGE results to the PulseNet database, and promptly interview all *Listeria* cases, particularly those among pregnant, Hispanic women, with the standard LI questionnaire (1). A supplemental questionnaire was administered to case patients to collect additional information about the consumption of 20 types of Mexican-style cheeses, the place of purchase, and brand information among cases reporting Mexican-style cheese consumption.

**Definitions.** Listeriosis was defined as illness in a person from whom a clinical specimen (cerebrospinal fluid, blood, or placenta) yielded *L. monocytogenes*. The novel two-enzyme PFGE pattern was designated as the outbreak pattern. For case counting, mother-infant pairs were considered a single case when the infant was less than 1 month old, regardless of whether one or both of the individuals in the mother-infant pair were ill with culture-confirmed *L. monocytogenes* infection.

**Laboratory investigation.** *L. monocytogenes* isolates recovered from case patients were confirmed by the AccuProbe DNA probe test (GenProbe), serotyped by standard methods (12), and subtyped by PFGE using *AscI* and *ApaI* restriction endonucleases following the PulseNet standardized protocol (4).

**Case-control study.** Cases were defined as illnesses among pregnant, Hispanic women with onsets from October 2008 to March 2009 and an *L. monocytogenes* isolate indistinguishable from the outbreak strain by PFGE with *AscI* and *ApaI*. Controls were defined as *L. monocytogenes* infections in non-outbreak-related pregnant, Hispanic women that were reported through the LI during 2004 to 2008. Cases and controls were interviewed with the standard LI questionnaire, which asks about symptoms, food consumption (covering over 40 items), and shopping histories during the month before illness onset, or before delivery date for cases in pregnant women.

**Analyses.** Odds ratios (ORs), *P* values, and 95% confidence intervals (CIs) were calculated using SAS software, version 9.2 (SAS Institute). A mid-*P* analysis for the *P* value and CI summary of the two tables was used in order to obtain comparable CIs for the two ORs (10). This was necessitated by the zero cell in the 2 × 2 table for Mexican-style cheese.

**Manufacturing plant inspection and testing.** The Michigan Department of Agriculture's Food and Dairy Division (MDA) routinely collects food and environmental samples from dairy manufacturing facilities in Michigan. MDA inspectors typically request that dairy products be tested for *L. monocytogenes* and/or *Salmonella* species at the MDA Geagley Laboratory. Samples that result in confirmed isolation of a pathogen typically prompt follow-up inspection and subsequent testing by MDA. *L. monocytogenes* isolates undergo PFGE analysis and are submitted to PulseNet for comparison with the national database.

**Traceback investigation.** The results of the case-control study and information gathered on the supplemental questionnaires were used to focus the product traceback investigation. Officials from state health and agricultural departments visited retail establishments where case patients reported purchasing Mexican-style cheese to determine if case patients could have obtained the cheese from a common source. Product samples obtained at the time of the investigation and invoices from products sold at the time of exposure were collected. Samples of Mexican-style cheese were cultured by standard methods (6).

## RESULTS

**Case finding and clinical syndrome.** Eight case patients were identified in five states (Illinois [3], Georgia [2], North Carolina [1], Tennessee [1], and Wisconsin [1]) with onset dates ranging from 22 October 2008 to 21 March 2009. Demographic and clinical data of ill persons are described in Table 1.

**Case-control study.** Seven case patients and 43 controls were enrolled in the case-control study. All case patients and controls were pregnant, Hispanic females. The case of a 3-year-old Hispanic girl was excluded from the case-control study because we were unable to find an appropriate ill control. In univariate analyses, case patients were more likely than controls to have consumed Mexican-style cheese during the month before illness onset (7 [100%] of 7 versus 26 [60%] of 43; OR = 5.89; 95% CI = 1.07 to ∞; *P* = 0.04) and fruit salad (5 [83%] of 6 versus 15 [35%] of 43; OR = 8.92; 95% CI = 1.11 to 229.11; *P* = 0.04) (5). Three (75%) of four case patients reported purchasing Mexican-style cheeses at deli counters. No other food items were significantly associated with illness.

**Plant inspection and testing.** On 23 February 2009, MDA performed a routine inspection of plant A and collected a sample of Mexican-style asadero cheese. Several days later, preliminary culture identified a possible *Listeria* strain; pending further investigation, cheese produced in the plant was not allowed to be distributed. In early March, *L. monocytogenes* was isolated from the sample, and MDA inspectors seized and destroyed 729 pounds of asadero cheese from plant A and forbade the distribution of all other cheeses produced in the plant. Five days later, inspectors collected an additional 23 environmental samples and two cheese samples from plant A; *L. monocytogenes* was isolated from a vat gasket in a postpasteurization section of the asadero cheese production line. The PFGE patterns of both isolates matched the pattern of the outbreak strain. Subsequently, MDA collected an additional 125 environmental, ingredient, and product samples over 2 days; none of these samples yielded *Listeria*. Records at the plant indicated that product manufactured on the same day as the positive asadero cheese sample was distributed to 10 states, including the 5 states with matching human isolates. On 23 March 2009 a recall was issued for asadero and Oaxaca cheeses manufactured in plant A for all production dates between 1 August 2008 and 27 February 2009.

State and federal regulators met with plant owners to discuss remediation steps in process flow, personnel, and

TABLE 1. Demographic and clinical features of ill persons with listeriosis due to the outbreak strain

Characteristic	Pregnancy associated		
	Neonate	Woman	Child
No. of individuals	5	7	1
Age range (median)	25–39 (34) wk of gestation	21–43 (31.5) yr	3 yr
No. (%) of females		7 (100)	1 (100)
No. (%) with Hispanic ethnicity	5 (100)	7 (100)	1 (100)
No. (%) with signs and symptoms of:			
Fever		3 (50)	1 (100)
Headache		2 (33)	1 (100)
Muscle aches		2 (33)	
Stiff neck		2 (33)	
Chills		1 (17)	
Vomiting		1 (17)	1 (100)
Other <sup>a</sup>		1 (17)	1 (100)
No. (%) of individuals with:			
<i>Listeria</i> bacteremia	3 (75)	2 (33)	0 (0)
Pneumonia	1 (25)		
<i>Listeria</i> meningitis	0 (0)	0 (0)	1 (100)
Other	0 (0)	2 (33)	0 (0)
None	0 (0)	2 (33)	0 (0)
No. (%) hospitalized	5 (100)	3 (50)	1 (100)
No. (%) with pregnancy outcome of:			
Live birth		5 (71)	
Stillbirth		2 (29)	

<sup>a</sup> Other signs and symptoms include nausea, abdominal pain or cramps, and unspecified vaginal infection; some cases reported more than one “other” symptom.

equipment sanitation. Employees were trained in proper personal hygiene practices for decontamination when moving between raw and finished product areas. After the plant changed employee traffic patterns and removed the asadero vat with the contaminated gasket, regulators approved the plant to begin production of soft Hispanic-style cheeses in April 2009. During the next month, state and Federal regulators sampled products and reviewed plant processes. In late May, a queso fresco sample collected by the U.S. Food and Drug Administration tested positive for *L. monocytogenes*, resulting in a second seizure of product by MDA and a voluntary recall of all Hispanic-style cheeses produced in the plant; MDA staff issued a cease and desist order for the production of these cheeses.

Federal regulators requested that the facility issue a Class I recall of all its semisoft cheeses and halt production of all cheeses. Plant A closed in June 2009 and remains closed. An assessment of the plant by MDA after closure and removal of all equipment showed an opening in the wall between the raw milk processing room and the finished product packaging room.

**Traceback investigation.** Case patients reported obtaining Mexican-style cheese from a variety of Hispanic markets, but one-half of them did not recall the specific brand names of the purchased cheese. Information regarding the place of purchase was available for seven cases; three of the patients purchased cheese from multiple locations. The Illinois Department of Public Health, Division of Foods,

Drugs, and Dairies traced product from plant A to the three stores where the Illinois cases were known to have purchased Mexican-style cheese. All three Illinois cases purchased Mexican-style cheese at different stores. Investigators collected 16 product samples from these locations from 13 to 27 January 2009; none yielded *Listeria*.

**Laboratory investigation.** *L. monocytogenes* isolates recovered from seven case patients and from four cheese samples and one environmental sample from plant A were serotype 1/2a and had indistinguishable PFGE patterns by use of *AscI* (GX6A16.0527) and *ApaI* (GX6A12.1603) restriction endonucleases.

## DISCUSSION

We report an outbreak of listeriosis that occurred mainly among pregnant, Hispanic women and was caused by consumption of commercial, Mexican-style cheese made from properly pasteurized milk. Case-control analysis identified Mexican-style cheese as the most likely source of the outbreak; this finding is supported by isolation of the outbreak strain of *L. monocytogenes* from a Mexican-style cheese product from plant A, and the distribution of this product to states with matching human isolates and to stores in Illinois where three case patients purchased Mexican-style cheeses.

The presence of *L. monocytogenes* in a cheese made from properly pasteurized milk likely resulted from postpasteurization contamination. A vat gasket emerged as

a possible source of contamination of pasteurized milk and indicates cleaning issues within the plant as well as potential gasket maintenance issues. We do not conclude that the gasket itself was the only source of contamination because cheese that was produced at the plant after removal of the vat with the contaminated gasket continued to test positive for *L. monocytogenes*. Plant inspection also detected a hole in the wall between the raw milk processing room and the cheese packaging room. *L. monocytogenes* was not isolated from the wall surrounding this hole, though findings from other investigations support this defect as a possible source of contamination of the postpasteurization processing room. Environmental reservoirs of *L. monocytogenes* associated with previous foodborne outbreaks include *L. monocytogenes* reservoirs in plant areas with moisture, such as drains, walls (especially if there are cracks that retain moisture), condensate, and wet insulation (14). During the investigation of one previous outbreak associated with *L. monocytogenes*—contaminated hot dogs, an inspection of the plant yielded *L. monocytogenes* from cinder blocks around an opening in a wall between the peeler room and the packaging room that allowed moisture to accumulate in the blocks (13).

This is the first report of a listeriosis outbreak in the United States to be associated with commercial, Mexican-style cheese made from properly pasteurized milk. However, in 1985, commercially produced Mexican-style cheese was implicated in an outbreak of listeriosis with 86 cases, including 58 mother-infant pairs, in Los Angeles County, California (8). In that outbreak, investigation of the cheese plant suggested that the pasteurized milk was routinely contaminated with unpasteurized milk.

The CDC PulseNet database contains PFGE patterns from >4,600 human *L. monocytogenes* isolates analyzed by both *AscI* and *ApaI* restriction endonucleases; this is the first time this particular *ApaI* pattern has been identified, making this pattern combination new to the database.

The case-control analysis also identified fruit salad as a possible source of the outbreak. Melons were a risk factor for listeriosis in a study of sporadic cases (15). However, all case patients reported consumption of Mexican-style cheese but not of fruit salad.

Our study is subject to several limitations. Information bias is possible given the long exposure period for dietary histories. A person answering questions about consumption patterns in the past month may respond based on dietary preferences, rather than what was actually eaten, as it may be difficult to remember the exact items eaten. Controls used in this study were persons with culture-confirmed *L. monocytogenes* infection that were reported to the LI; exposure information collected on all cases of *L. monocytogenes* is based on the month prior to onset, and thus, controls were interviewed before cases occurred and consumption information for controls in this study is for a time period that is different from the period for the case patients. The small number of pregnant, Hispanic women reported to the LI prevented matching cases by age, state, and trimester of pregnancy; the small number of cases limited the power of the study. This outbreak likely

involved additional illnesses because approximately one-half of *L. monocytogenes* infections in the United States are detected by public health surveillance systems (11). Cases and controls may have been overmatched by limiting controls to those of Hispanic ethnicity; use of all pregnant women as controls would have shown a much higher association with Mexican-style cheese.

Cheese manufacturers and dairies should ensure that plant design and traffic patterns help to minimize the possibility of contamination of finished dairy products. Additionally, cheese manufacturers and dairies should maintain high standards of plant sanitation through adequate cleaning, sanitizing, and maintenance of equipment and monitor their final products for pathogens such as *L. monocytogenes*. Consumers of pasteurized dairy products should store them at 4°C or lower and consume the products before their expiration dates (3). The growing Hispanic population in the United States and the high frequency of consumption of Mexican-style cheese among pregnant, Hispanic *Listeria* case patients highlight the importance of continued regulatory efforts, such as routine plant inspections.

Rapid interviewing of case patients, integration of epidemiologic and laboratory data from the LI, routine food regulatory inspections, and PulseNet led to rapid identification of the source of the outbreak and regulatory action to prevent additional illnesses. The LI provided timely, appropriate study controls that were matched to case patients by pregnancy status and ethnicity; these controls would have been extremely difficult to identify through typical control-finding methods such as random digit dialing. Although the LI case-control study showed an association with Mexican-style cheese, identification of Mexican-style cheese as the most likely source vehicle might not have occurred without the independent, routine inspection of plant A by MDA because of patients' limited recall of brand information. Therefore, this investigation highlights the added value of integrating epidemiologic and food regulatory data through national surveillance systems like PulseNet.

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## REFERENCES

1. Centers for Disease Control and Prevention. 15 March 2010. Listeriosis surveillance and the *Listeria* Initiative. Available at: [http://www.cdc.gov/nationalsurveillance/listeria\\_surveillance.html](http://www.cdc.gov/nationalsurveillance/listeria_surveillance.html). Accessed 27 April 2010.
2. Centers for Disease Control and Prevention. 18 November 2009. Listeriosis: general information. Available at: <http://www.cdc.gov/nczved/divisions/dfbmd/diseases/listeriosis/>. Accessed 27 May 2010.
3. Dalton, C., C. Austin, J. Sobel, P. Hayes, W. Fibb, L. Graves, B. Swaminathan, M. Proctor, and P. M. Griffin. 1997. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. *N. Engl. J. Med.* 336:100–105.
4. Graves, L. M., and B. Swaminathan. 2001. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *Int. J. Food Microbiol.* 65: 55–62.

5. Hirji, K. F., A. A. Tsiatis, and C. R. Mehta. 1989. Median unbiased estimation for binary data. *Am. Stat.* 43:7–11.
6. Hitchins, A. D. 14 May 2009. Detection and enumeration of *Listeria monocytogenes* in foods. In *Bacteriological analytical manual*. Available at: <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM071400#authors>. Accessed 1 February 2011.
7. Jackson, K. A., M. Iwamoto, and D. Swerdlow. 2010. Pregnancy-associated listeriosis. *Epidemiol. Infect.* 138:1503–1509.
8. Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, P. Hayes, and R. Weaver. 1988. Epidemic listeriosis associated with Mexican-style cheese. *N. Engl. J. Med.* 319:823–828.
9. MacDonald, P. M. D., R. E. Whitwam, J. D. Boggs, J. N. McCormack, K. L. Anderson, J. W. Reardon, J. R. Saah, L. M. Graves, S. B. Hunter, and J. Sobel. 2005. Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese. *Clin. Infect. Dis.* 40:677–682.
10. SAS. 2010. The LOGISTIC procedure. SAS, Cary, NC.
11. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.
12. Seeliger, H., and K. Hohne. 1979. Serotyping of *Listeria monocytogenes* and related species. *Methods Microbiol.* 13:31–49.
13. Tompkin, R. B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. *J. Food Prot.* 65:709–725.
14. Tompkin, R. B., V. N. Scott, D. T. Bernard, W. H. Sveum, and K. S. Gombas. 1999. Guidelines to prevent post-processing contamination from *Listeria monocytogenes*. *Dairy Food Environ. Sanit.* 19:551–562.
15. Varma, J. K., M. C. Samuel, R. Marcus, R. M. Hoekstra, C. Medus, S. Segler, B. J. Anderson, T. F. Jones, B. Shiferaw, N. Haubert, M. Megginson, P. V. McCarthy, L. Graves, T. V. Gilder, and F. J. Angulo. 2007. *Listeria monocytogenes* infection from foods prepared in a commercial establishment: a case-control study of potential sources of sporadic illness in the United States. *Clin. Infect. Dis.* 44: 521–528.