

AN OUTBREAK OF GASTROENTERITIS AND FEVER DUE TO *LISTERIA MONOCYTOGENES* IN MILK

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ABSTRACT

Background After an outbreak of gastroenteritis and fever among persons who attended a picnic in Illinois, chocolate milk served at the picnic was found to be contaminated with *Listeria monocytogenes*.

Methods In investigating this outbreak, we interviewed the people who attended the picnic about what they ate and their symptoms. Surveillance for invasive listeriosis was initiated in the states that receive milk from the implicated dairy. Stool and milk samples were cultured for *L. monocytogenes*. Serum samples were tested for IgG antibody to listeriolysin O.

Results Forty-five persons had symptoms that met the case definition for illness due to *L. monocytogenes*, and cultures of stool from 11 persons yielded the organism. Illness in the week after the picnic was associated with the consumption of chocolate milk. The most common symptoms were diarrhea (present in 79 percent of the cases) and fever (72 percent). Four persons were hospitalized. The median incubation period for infection was 20 hours (range, 9 to 32), and persons who became ill had elevated levels of antibody to listeriolysin O. Isolates from stool specimens from patients who became ill after the picnic, from sterile sites in three additional patients identified by surveillance, from the implicated chocolate milk, and from a tank drain at the dairy were all serotype 1/2b and were indistinguishable on multilocus enzyme electrophoresis, ribotyping, and DNA macrorestriction analysis.

Conclusions *L. monocytogenes* is a cause of gastroenteritis with fever, and sporadic cases of invasive listeriosis may be due to unrecognized outbreaks caused by contaminated food. (N Engl J Med 1997; 336:100-5.)

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THERE was an outbreak of gastroenteritis and fever among the people who ate at a picnic at a Holstein cow show in Elizabeth, Illinois, on July 9, 1994. Complaints about the taste and quality of commercial pasteurized chocolate milk consumed at the picnic led to the culture of *Listeria monocytogenes* from samples of leftover milk. Milk with the same production date from the same dairy had been distributed throughout Wisconsin and part of Michigan. In the past, outbreaks caused by food-borne *L. monocytogenes*

have generally been characterized by severe invasive disease.¹⁻⁴ However, in recent outbreaks, infection with *L. monocytogenes* has presented with fever and gastroenteritis without progression to invasive illness.^{5,6} We investigated the cause and nature of illness among attendees at the picnic in Illinois and initiated surveillance to determine whether *L. monocytogenes* in chocolate milk had also caused illness in Wisconsin and Michigan.

METHODS

Epidemiologic Investigation

Efforts were made to contact all persons who had gone to the picnic. Using a standard questionnaire, we interviewed attendees by telephone or in person about all the foods and beverages they consumed at the picnic and about the quantity of chocolate milk they consumed and its temperature and taste. Information about the frequency and duration of symptoms, use of medications, and medical history was also obtained. To analyze risk factors for illness and to describe the relation between illness and serologic response, we defined a case of *L. monocytogenes* infection as the presence of symptoms from two of the following four groups within one week after the consumption of foods or beverages at or from the picnic: (1) fever; (2) diarrhea (defined as loose stools for 24 hours or more), nausea, or vomiting; (3) myalgia or arthralgia; and (4) headache.

Environmental and Laboratory Investigation

All the people who attended the picnic were asked to submit stool and blood specimens. Rectal swabs were transported in refrigerated Cary-Blair medium to the Centers for Disease Control and Prevention (CDC) in Atlanta, where they were transferred to U.S. Department of Agriculture listeria enrichment broth (modified University of Vermont [UVM] formulation).⁷ Stool samples (1.0 g) were inoculated into listeria enrichment broth and transported at ambient temperature to the CDC. Listeria was isolated by the method of McClain and Lee.⁷ Suspect colonies were streaked onto trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with 5 percent sheep's blood and incubated for 18 hours at 35°C. If the isolates were confirmed to be *L. monocytogenes*,⁸ we determined the serotype.⁹ Isolates of the serotype responsible for the Illinois outbreak were further characterized by multilocus enzyme electrophoresis,¹⁰ ribotyping,¹¹ and DNA macrorestriction analysis with use of pulsed-field gel

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electrophoresis.^{12,13} *L. monocytogenes* in the chocolate milk was measured by direct plating.¹⁴

Stool specimens from five picnic attendees were also examined for shigella, salmonella, campylobacter, pseudomonas, vibrio, *Escherichia coli* O157:H7,¹⁵ *Yersinia enterocolitica*, *Clostridium perfringens*, enterotoxigenic *E. coli*, and coliforms; stool specimens from 11 attendees were examined for *Bacillus cereus* and *Staphylococcus aureus*.¹⁶ Chocolate milk was cultured for *L. monocytogenes*, shigella, salmonella, campylobacter, *B. cereus*, *C. perfringens*, yersinia, pseudomonas, *E. coli* O157:H7, and coliforms at the CDC.¹⁶

Antibodies against listeriolysin O were detected with an enzyme-linked immunosorbent assay (ELISA)¹⁷ based on listeriolysin O purified by affinity chromatography with a monoclonal-antibody ligand attached to a solid matrix.¹⁸ Serum samples from 51 adults with other enteric infections were used as controls.

Illness Detected by Surveillance

Health departments in Wisconsin, Illinois, and Michigan conducted surveillance for febrile gastrointestinal illness associated with the consumption of chocolate milk from the implicated dairy and for invasive listeriosis by issuing press releases and memoranda to all infection-control practitioners, laboratory directors, and local health departments.

Statistical Analysis

Statistical analysis was performed with EpiInfo version 6.02,¹⁹ except for the calculation of lower 95 percent confidence limits, for which StatXact 3 software was used.²⁰ Chi-square tests were used for the analysis of categorical variables. The Wilcoxon two-sample test was used to compare median anti-listeriolysin O levels between patients with illness that met the case definition and controls.

RESULTS

Epidemiologic Investigation

Eighty-two (89 percent) of the estimated 92 persons who attended the picnic were interviewed. The median age of those who had an illness that met the case definition was 31 years (range, 3 to 79); the median age of those who did not have such an illness was 24 years (range, 4 to 69). Nineteen of 37 females (51 percent) met the case definition, as compared with 26 of 45 males (58 percent). None of the people who went to the picnic reported having a chronic illness or immune deficiency. One woman was 40 weeks pregnant; the day after the picnic she had a six-hour episode of diarrhea, with no other symptoms. She delivered a healthy baby five days later.

Forty-five (75 percent) of the 60 people who consumed chocolate milk at the picnic reported an illness that met the case definition, as compared with none of the 22 persons who did not drink chocolate milk (Table 1). Nine other persons who consumed the implicated milk at the picnic had an illness in the week after the picnic that did not meet the case definition, indicating that up to 54 (90 percent) of the 60 picnic attendees who consumed chocolate milk may have become ill as a result. The only other food associated with illness was Swiss cheese; however, only 25 persons ate this, and they all drank chocolate milk as well. Drinking plain milk was slightly protective (relative risk of illness among those who

TABLE 1. FOOD-SPECIFIC ATTACK RATES AMONG PERSONS WHO ATTENDED THE PICNIC AT THE HOLSTEIN COW SHOW IN ILLINOIS, JULY 1994.*

FOOD ITEM	PERSONS WHO CONSUMED THE ITEM		PERSONS WHO DID NOT CONSUME THE ITEM		RELATIVE RISK (95% CI)
	NO. WITH ILLNESS/ TOTAL NO.	ATTACK RATE (%)	NO. WITH ILLNESS/ TOTAL NO.	ATTACK RATE (%)	
Chocolate milk	45/60	75	0/22	0	Undefined (13.6-∞)
Plain milk	10/20	50	33/60	55	0.9 (0.6-1.5)
Swiss cheese	21/25	84	14/43	33	2.6 (1.6-4.1)
Colby cheese	24/38	63	7/17	41	1.5 (0.8-2.9)
Cheddar cheese	16/25	64	15/31	48	1.3 (0.8-2.1)
Roast beef	31/52	60	12/27	44	1.4 (0.8-2.2)
Ham	27/47	57	15/31	48	1.2 (0.8-1.8)
Potato chips	37/66	56	8/15	53	1.1 (0.6-1.8)
Buns	38/65	58	7/17	41	1.4 (0.8-2.6)

*For each food item, only the persons for whom information on consumption was available are included. Relative risks are the risk of illness among those who consumed a food item, as compared with the risk among those who did not consume it. CI denotes confidence interval.

drank plain milk as compared with those who did not, 0.9). Among the four people who did not attend the picnic but drank chocolate milk brought home from the picnic, three had an illness meeting the case definition. Among the people who provided information on how much chocolate milk they consumed, there was no difference in the amount consumed by 42 people who became ill and 15 who did not; both groups consumed a median of one 8-oz (240-ml) carton (range, one to eight).

Among the 58 people who drank milk from the picnic (either at the picnic or thereafter) and reported any symptoms in the seven days after consuming the milk, 50 percent or more had each of the following: diarrhea, fatigue, fever, chills, headache, myalgia, and abdominal cramps (Table 2). Diarrhea lasted a median of 42 hours, and fever a median of 27 hours. Persons with diarrhea had a median of 12 stools (range, 3 to 50) during the 24 hours of maximal diarrhea. Among the 17 persons whose temperature was reported, the median maximal temperature was 38.9°C (range, 37.8 to 40.3°C). The median incubation period (the time to the first symptom included in the case definition) was 20 hours (range, 9 to 32) for the 48 persons whose illness met the case definition (including 3 who consumed the chocolate milk at home). Four persons — 1, 7, 49, and 77 years of age — were hospitalized for a total of eight hospital days; none of them died.

Environmental Investigation

Most of the cartons of chocolate milk served at the picnic on July 9 were marked with an expiration date

TABLE 2. PREVALENCE OF VARIOUS SYMPTOMS AMONG THE 58 PERSONS WHO REPORTED SYMPTOMS AFTER CONSUMING CHOCOLATE MILK FROM THE PICNIC AT THE HOLSTEIN COW SHOW.

SYMPTOM	PREVALENCE (%)
Diarrhea	79
Fatigue	74
Fever	72
Chills	65
Headache	65
Myalgia	59
Abdominal cramps	55
Nausea	47
Vomiting	26
Arthralgia	25
Sore throat	3
Bloody diarrhea	3

of July 12. A total of 5600 8-oz cartons of chocolate milk containing 1 percent fat were manufactured on June 24, with an expiration date of July 12. Chocolate flavoring was added before the milk was pasteurized at 87°C or higher for 18 seconds; the milk was immediately cooled to less than 8°C. No defects in the pasteurization process were identified. The milk was pumped to a holding tank that was designed to be refrigerated; however, the poor condition of the insulating jacket had not allowed the use of a refrigerant for three years. Inspection revealed a breach in the lining that allowed milk to leak into the insulation jacket, creating a pool of sequestered milk. As the tank was drained, sequestered milk could reenter through the breach. The sanitizing-solution sprayers were severely clogged, inhibiting the flow of sanitizing solution onto the tank lining.

The pasteurized chocolate milk was held in the unrefrigerated tank for two hours, then pumped over the next seven hours into a machine that filled and sealed half-pint containers. On the day of production, there was a single handwritten temperature recording of 45°F (7.2°C) for a randomly sampled carton of chocolate milk as it left the filler. The cartons were stored in a refrigerated room until they were transported in a refrigerated truck to a distributor in Madison, Wisconsin. A company employee reported that on the day before the picnic, 180 8-oz cartons of chocolate milk produced on June 24 and 60 8-oz cartons of plain milk were transported without refrigeration for 2¼ hours to Elizabeth, Illinois. The milk was then placed in one domestic refrigerator. The next morning, at approximately 11 a.m., the milk was placed in an unrefrigerated cooler and transported to the picnic site. Most of it was consumed between 11:30 a.m. and 12:30 p.m., but it

remained available and unrefrigerated throughout the afternoon.

Laboratory Investigation

Milk Specimens

L. monocytogenes was isolated from multiple unopened products from the dairy, including 8-oz cartons of chocolate milk with a production date of June 24 that were left from the picnic and chocolate milk with the same production date that was not sent to the picnic. Two unopened cartons of chocolate milk, one from the picnic and one produced on the same day at the dairy, yielded 1.2×10^9 and 8.8×10^8 colony-forming units (CFU) of *L. monocytogenes* per milliliter, respectively. Since the median volume consumed at the picnic by those who drank chocolate milk was 8 oz, the median dose of listeria may have been as high as 2.9×10^{11} CFU per person.

Environmental Cultures

L. monocytogenes was isolated from 2 of 64 environmental specimens from the dairy. One was obtained on July 19 from a floor drain beneath the chocolate-milk filler, and the other was obtained on July 22 from a valve connected to the chocolate-milk pasteurizer. None of the six specimens obtained on July 22 from the empty post-pasteurization holding tank yielded listeria.

Cultures of Samples from Patients

Stool specimens were collected from 41 persons who consumed chocolate milk from the picnic; specimens from 38 (93 percent) were collected from 16 to 19 days after the consumption of the milk. *L. monocytogenes* was isolated from the stools of 11 persons. People with more severe illness were more likely to have positive stool cultures. Among the people who consumed chocolate milk, stool cultures were negative in 6 who had had no symptoms, positive in 11 (37 percent) of the 30 whose illness met the case definition, and positive in 3 (75 percent) of the 4 who were hospitalized. The stool specimens did not yield other pathogens, except for one specimen that yielded 3×10^6 CFU of *Clostridium perfringens* per gram. Cultures of blood from a hospitalized person whose illness met the case definition were negative.

Subtyping

L. monocytogenes isolates from the 11 positive stool samples, from chocolate milk, and from the floor drain and vacuum valve at the dairy were all serotype 1/2b and were indistinguishable from each other by multilocus enzyme electrophoresis (all were enzyme type 7) and by ribotyping with *EcoRI* restriction-enzyme analysis. When the same isolates were evaluated for clonality by pulsed-field gel elec-

trophoresis, all but one had identical restriction patterns; one patient's isolate differed by a single band from the others (Fig. 1).

Serologic Results

Forty-eight people who had been at the picnic submitted blood for serologic testing a median of 18 days after consuming chocolate milk (range, 16 to 20 days). The median anti-listeriolysin O level among picnic attendees was 57 ELISA units (an arbitrary measure) in 9 persons who were asymptomatic, 92 units in 8 persons with mild illness, and 143 units in 31 persons with illness that met the case definition (Fig. 2). The median anti-listeriolysin O level of the 51 control samples was 63 ELISA units, significantly lower than the median for the patients who had an illness that met the case definition ($P < 0.001$).

Illness Detected by Surveillance

In July, a febrile gastrointestinal illness similar to the illness in the outbreak developed in all the members of an Illinois family of five (who did not attend the picnic) after they consumed chocolate milk from the implicated dairy while traveling through Michigan. All five submitted stool specimens to the CDC. *L. monocytogenes* 1/2b, enzyme type 7, with the same ribotype and the same pattern on pulsed-field gel electrophoresis as the strain in the outbreak was cultured from one family member's stool. Also in July, 20 Wisconsin residents reported having gastrointestinal illness after consuming the implicated milk; cultures for *L. monocytogenes* were negative, however.

Between July 1 and September 30, 1994, 27 isolates of *L. monocytogenes* from blood or cerebrospinal fluid were identified in Wisconsin (2 isolates), Michigan (11 isolates), and Illinois (14 isolates). Of the nine isolates sent to the CDC for serotyping, three were serotype 1/2a, three were serotype 1/2b, and three were serotype 4b.

All three isolates of serotype 1/2b were obtained in July. All were enzyme type 7 and were indistinguishable from the strain identified in the outbreak at the Illinois picnic according to ribotyping and pulsed-field gel electrophoresis. One was isolated from the blood of a two-year-old Michigan girl. She had consumed chocolate milk in the week before the onset of her illness at a restaurant that served milk from the implicated dairy. Another isolate was obtained from a cerebral abscess in a 72-year-old Wisconsin man. Approximately three weeks before hospitalization, he had had fever, diarrhea, chills, vomiting, myalgia, joint aches, headaches, and fatigue. He had a history of bladder cancer and ischemic heart disease but was not taking immunosuppressive medication. He had consumed chocolate milk from the implicated dairy an average of once each week in the two months before he became ill.

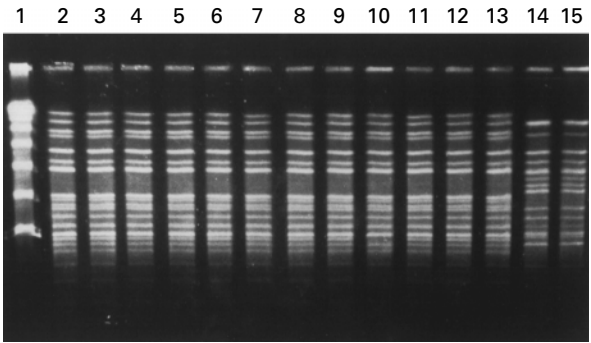


Figure 1. DNA Macrorestriction Patterns of *Listeria monocytogenes* Isolates, Determined by *Apal* Restriction of Genomic DNA and Separation of the Restriction Fragments by Pulsed-Field Gel Electrophoresis.

A CHEF-DR II system (Bio-Rad Laboratories, Hercules, Calif.) was used for the electrophoresis. Lane 1 shows the molecular-size standards (λ ladder); lanes 2 to 11 show isolates from stool specimens from persons with noninvasive listeriosis; lanes 12 and 13, isolates from the implicated chocolate milk; and lanes 14 and 15, isolates of serotype 1/2b unrelated to this outbreak. Note the absence of a single band in lane 10, just below the bright band corresponding to the bottom edge of the lowest fragment in lane 1.

His anti-listeriolysin O level after discharge from the hospital was 382 ELISA units. The third isolate was from the blood of an 81-year-old Wisconsin resident; an undetermined length of time before the onset of diarrhea and fever, he had consumed chocolate milk purchased from a store that sold milk from the implicated dairy.

DISCUSSION

This outbreak was most likely caused by post-pasteurization contamination due to poor sanitation practices at the milk company and exacerbated by holding temperatures in transit to the picnic that allowed the rapid growth of listeria.^{21,22} Our data strongly suggest that both the noninvasive illness among the picnic attendees and at least three cases of invasive illness were caused by the same strain of *L. monocytogenes*. There was a strong association between drinking chocolate milk contaminated with *L. monocytogenes* and becoming ill. The chocolate milk contained very high levels of *L. monocytogenes*. No other pathogen compatible with the illness suffered by the picnic attendees was present in the chocolate milk or in the stools of ill persons. All isolates from ill persons who had attended the picnic, from the three persons with cases of invasive listeriosis detected by surveillance, and from the implicated chocolate milk were enzyme type 7, with the same ribotype, and — with one exception — were indistinguishable from each other by DNA macrorestriction analysis. The link between invasive illness

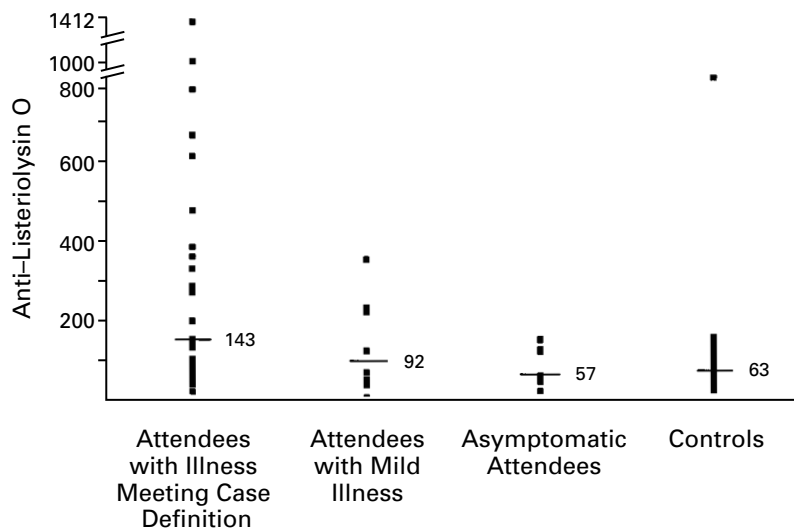


Figure 2. Anti-Listeriolysin O Levels among Picnic Attendees and Controls.

Among the 48 persons whose blood was tested, 31 had an illness that met the case definition, 8 had mild illness, and 9 were asymptomatic; there were 51 controls with other enteric infections. The horizontal lines indicate the median anti-listeriolysin O levels. Anti-listeriolysin O levels are expressed in arbitrary ELISA units.

and the implicated milk was further confirmed in Wisconsin by pulsed-field gel electrophoresis.²³ During active surveillance for listeriosis in the United States in 1986 and 1987, only 14 (6.3 percent) of 222 isolates of *L. monocytogenes* found by surveillance and sent to the CDC were enzyme type 7 (these isolates accounted for 16.9 percent of serotype 1/2b isolates).¹⁰ Similar results were obtained in tests of isolates submitted to the CDC in 1994 (Bibb WF: unpublished data). Ill persons were more likely than well persons to have elevated anti-listeriolysin O levels and to have stool cultures that yielded *L. monocytogenes*. In addition, the incubation period and some features of the clinical illness are similar to cases of *L. monocytogenes* infection induced experimentally in nonhuman primates and goats during feeding trials.^{24,25}

Several other studies have suggested that fever and gastroenteritis are important features of infection with *L. monocytogenes* and, in some patients, can be the sole manifestation of infection. A prodromal illness characterized by fever and, occasionally, by vomiting, diarrhea, and abdominal pain has been reported in nonpregnant adults with *L. monocytogenes* bacteremia.²⁶ In an outbreak of invasive listeriosis in Philadelphia in 1986 and 1987, 35 percent of patients had diarrhea, 27 percent had vomiting, and 52 percent had fever in the week before their positive cultures.²⁷ Gastrointestinal symptoms and fever were commonly reported by persons with noninvasive disease in an outbreak of listeriosis in Connecticut; however, the median incubation period for

noninvasive illness was 21 days, and only one person with noninvasive illness had a positive stool culture.⁵ In an outbreak among immunocompetent adults, the most common presentation was gastrointestinal illness with a median incubation period of 18 hours; however, no stool specimens collected from patients with illness yielded *L. monocytogenes*.⁶

The noninvasive illness described by the people who attended the picnic was not benign. Many persons reported being bedridden and losing time from work, and four were hospitalized. The one blood culture that was obtained was negative; however, it is possible that the patients had transient bacteremia. Recovery from *L. monocytogenes* bacteremia without antibiotic treatment has been well documented.^{26,28}

The serologic test for anti-listeriolysin O is a relatively new and promising test that may assist in the diagnosis of both invasive and noninvasive listeriosis. Berche et al. found antibodies to listeriolysin O in 27 of 28 patients with invasive listeriosis.¹⁷ In the outbreak described here, a single high anti-listeriolysin O level during the convalescent phase was strongly associated with illness. This study demonstrates the potential value of this assay in the investigation of outbreaks of febrile gastroenteritis. A new method based on an amino-terminal residue of listeriolysin O may be even more specific for listeriosis.²⁹

Since *L. monocytogenes* is not detected by routine stool cultures, it may be a more common cause of febrile gastroenteritis than is currently recognized.

L. monocytogenes is often isolated from food³⁰ and can be isolated from the stools of 1 to 10 percent of healthy persons.^{4,31} Further studies are needed to determine the infectious dose and the characteristics of the host that are associated with noninvasive febrile gastroenteritis caused by *L. monocytogenes*.

Early recognition of outbreaks of gastroenteritis will allow commercial products contaminated with *L. monocytogenes* to be identified and recalled so as to prevent further gastrointestinal illness and possible invasive listeriosis. Clinicians and public health personnel should request that stools be cultured for *L. monocytogenes* in outbreaks of illness characterized by diarrhea, fever, headache, myalgia, and abdominal cramps, after the presence of other enteric pathogens has been ruled out. To prevent illness, dairies should monitor their final products for spoilage and for pathogens, including *L. monocytogenes*. Expiration dates should be based on the results of these tests and the general level of sanitation at the plant. High standards of plant sanitation should be maintained through adequate cleaning, sanitizing, and maintenance of equipment guided by a hazard-analysis and critical-control-point program. Consumers should store pasteurized dairy products at 4°C or at lower temperatures and should consume the products before their expiration dates.

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