



MARK B HORTON, MD, MSPH
Director

State of California – Health and Human Services Agency
California Department of Public Health



ARNOLD SCHWARZENEGGER
Governor

Date: March 27, 2008

To: The Record

From: Infectious Diseases Branch
Division of Communicable Disease Control
19300 S. Hamilton Ave., Suite 140
Gardena, CA 90248

Subject: CA EPI 08-03: Cluster of *Campylobacter* infections possibly associated with raw dairy products

We are pleased to provide the attached report on this investigation undertaken by Infectious Diseases Branch staff in collaboration with the California Department of Public Health Division of Communicable Disease Control Microbial Diseases Laboratory, Division of Food, Drug, and Radiation Safety, Food and Drug Branch, Food and Drug Laboratory Branch, the California Department of Food and Agriculture, the United States Department of Agriculture, Agriculture Research Service, and several local health departments.

Akiko C. Kimura, M.D.
Disease Investigations Section

Jeffrey I. Higa, M.P.H.
Disease Investigations Section

Duc J. Vugia, M.D., M.P.H., Chief
Infectious Diseases Branch

cc: Gilberto Chavez, M.D., M.P.H.
Deputy Director, State Epidemiologist
Center for Infectious Diseases
California Department of Public Health
1616 Capitol Ave. MS 7300
Sacramento, CA 95814

Douglas L. Hatch, M.D., M.P.H.
Division Chief
Division of Communicable Disease Control
California Department of Public Health
1616 Capitol Ave., MS-7300
Sacramento, CA 95814

Farzaneh Tabnak, M.S., Ph.D., Chief
Surveillance and Statistics Section
California Department of Public Health
1616 Capitol Ave. MS-7306
Sacramento, CA 95814

Ben Sun, D.V.M, M.P.V.M., Chief
Veterinary Public Health Section
California Department of Public Health
1616 Capitol Ave. MS-7307
Sacramento, CA 95814

Vicki Kramer, Ph.D., Chief
Vector Borne Disease Section
California Department of Public Health
1616 Capitol Ave. MS-7308
Sacramento, CA 95814

Debra Gilliss, M.D., M.P.H., Chief
Bioterrorism Epidemiology Section
California Department of Public Health
850 Marina Bay Parkway, Bldg. P., 2nd Floor
Richmond, CA 94804

Muntu Davis, M.D., Deputy
Communicable Disease Control Officer
Alameda County Public Health Department
1000 Broadway, Suite 500
Oakland, CA 94607

Francie Wise
Communicable Disease Control Officer
Contra Costa Public Health Division
597 Center Avenue, Suite 200A
Martinez, California 94553

Ken Bird, M.D.
Communicable Disease Control Officer
Fresno County Department of Community Health
1221 Fulton Mall
Fresno, CA 93721

Laurene Mascola, M.D.
Communicable Disease Control Officer
Los Angeles County Dept of Health Services
313 N. Figueroa Street, #909
Los Angeles, CA 90012

Linda Ferguson, P.H.N.
Communicable Disease Control Officer
County of Marin
899 Northgate Drive, Suite 100
San Rafael, CA 94903

Hildy Meyers, M.D.
Communicable Disease Control Officer
Orange County Health Care
405 W 5th, 7th floor
Santa Ana, CA 92702

Mark Starr, D.V.M.
Communicable Disease Control Officer
Placer County
11484 B Avenue
Auburn, CA 95603

Barbara Cole, R.N., P.H.N., M.S.N.
Communicable Disease Control Officer
Riverside County Dept of Public Health
P.O. Box 7600
Riverside, CA 92513-7600

Vivian E. Belmusto, M.D.
Communicable Disease Control Officer
Sacramento County Division of Public Health
7001-A East Parkway, Suite 600
Sacramento, CA 95823

Sara H. Cody, M.D.
Communicable Disease Control Officer
Santa Clara County Dept of Public Health
976 Lenzen Avenue
San Jose, CA 95126

Michael Stacey, M.D.
Communicable Disease Control Officer
Solano County Health and Social Services
275 Beck Avenue, MS 52-40
Fairfield, CA 94590

John Walker, M.D.
Communicable Disease Control Officer
Stanislaus County Health Services Agency
820 Scenic Drive
Modesto, CA 95350

Elise Osvold-Doppelhauer, P.H.N.
Communicable Disease Control Officer
Trinity County Health & Human Services
P.O. Box 1470
Weaverville, CA 96093

Jeff Farrar, D.V.M., Ph.D., M.P.H., Chief
Food and Drug Branch
California Department of Public Health
1500 Capitol Avenue - MS 7602
P.O. Box 997413
Sacramento, CA 95899-7413

Stephen Beam, Ph.D., Chief
Milk and Dairy Food Safety
California Department of Food and Agriculture
1220 N Street
Sacramento, CA 95814

To The Record
Page 5
March 27, 2008

John Michael Janda, Ph.D., Chief
Microbial Diseases Laboratory Branch
850 Marina Bay Parkway, Room E164
Richmond, CA 94804

Mary Soliman, Ph.D., MPH, Chief
Food & Drug Laboratory Branch
850 Marina Bay Parkway, G365
Richmond, CA 94804-6403

Robert E. Mandrell, Ph.D., Research Leader
Produce Safety and Microbiology Research Unit
USDA, ARS, WRRC
800 Buchanan Street
Albany, CA 94710

Summary

In December 2007, eight persons with *Campylobacter* infection who reported drinking a commercially available (Dairy A) raw dairy product were identified. Illness onsets were tightly clustered between late November and early December. Only one patient isolate was available for DNA fingerprinting; the pulsed-field gel electrophoresis patterns of isolates from four cattle fecal samples collected at Dairy A were indistinguishable from the patient isolate. Dairy A raw dairy products were highly suspect vehicles as the source of these illnesses, but further investigation to definitively implicate this product was not feasible.

Introduction

On December 10, 2007, the Orange County Health Care Agency reported to the California Department of Public Health (CDPH), Infectious Diseases Branch (IDB) two cases of febrile gastroenteritis possibly associated with commercial (Dairy A) raw milk or colostrum consumption. The cases were siblings aged 6 and 8 years old who developed fever and bloody diarrhea on November 26 and 27, 2007, respectively. Stool specimen collected December 3 from one child yielded *Campylobacter*; stool specimen from the other child was negative. The two children started drinking Dairy A raw whole milk about three weeks prior to onset of illness and drank the milk three times per day. In addition, they started drinking Dairy A raw colostrum three times per week about 10 days prior to illness onset. The raw milk, colostrum, and containers were no longer available for testing. Both products had been purchased from a commercial co-op.

CDPH implemented enhanced surveillance, inspected Dairy A, and conducted a laboratory investigation to: 1) identify additional cases and 2) determine if cases were associated with Dairy A raw dairy product exposure.

Methods

Enhanced Surveillance

For the purposes of case-finding, a confirmed case-patient was defined as a person with culture-confirmed *Campylobacter* infection with a history of Dairy A raw dairy product exposure in the week before illness onset. A suspect case-patient was defined as a person with gastrointestinal symptoms with a history of Dairy A raw dairy product exposure in the week before illness onset who was not culture confirmed (either negative or not tested).

On December 12, 2007, CDPH IDB sent an email to the California Conference of Local Health Officers (CCLHO) and communicable disease (CD) controllers requesting that any persons with campylobacteriosis or other enteric gastroenteritis (such as

Salmonella or *E. coli* O157:H7 infection) with exposure to raw milk or colostrum prior to onset of illness identified through routine surveillance, be immediately reported to IDB.

On December 20, 2007, CDPH IDB sent a second email to CCLHO and CD controllers requesting that their health departments interview all patients with *Campylobacter* infection with culture submission dates on or after December 1, 2007, using a standard questionnaire. The questionnaire asked about history of illness, travel, and exposure to sources previously associated with *Campylobacter* infection, including poultry, raw seafood, raw milk, and pets. In addition, the local health departments were requested to collect raw dairy products from the patient's home and case-patient isolates whenever available for additional testing.

On January 8, 2008, CDPH IDB sent a third email to CCLHO and CD controllers advising them to return to their routine *Campylobacter* surveillance.

Environmental Inspection

Dairy A is a licensed raw milk dairy located in Fresno County. On December 17, 2007, the CDPH Food and Drug Branch collected environmental samples from Dairy A including raw milk and raw colostrum, environmental swabs of milking equipment, milking barn discharge water, well water, and fresh cattle manure from the pasture. Additionally, retail samples produced by Dairy A, including raw milk and raw skim milk (best by 12/25/07), raw chocolate colostrum (best by 12/18/07), and raw butter (best by 12/31/07), were purchased from a commercial co-op for testing. These retail products were not from the same stores or time period as those consumed by the patients.

In addition, the California Department of Food and Agriculture (CDFA) reviewed the standard plate counts of product samples from the dairy in the three months prior to the period of interest (September-November 2007).

Laboratory Investigation

CDPH Microbial Diseases Laboratory (MDL) performed pulsed-field gel electrophoresis (PFGE) analysis on the one *Campylobacter jejuni* isolate available from a case-patient. In addition, CDPH MDL performed PFGE analysis from a selected number of *Campylobacter* cases with no history of raw dairy product exposure to serve as controls.

The Food and Drug Laboratory Branch (FDLB) and the United States Department of Agriculture, Agriculture Research Service (USDA ARS) cultured *C. jejuni* from environmental samples collected at Dairy A. USDA ARS screened the positive cultures utilizing the *C. jejuni* major outer membrane protein (MOMP) gene and multilocus sequence typing (MLST) to identify the outbreak strain in environmental samples. A selected number of isolates were sent to MDL to compare with the case-patient isolate.

Results

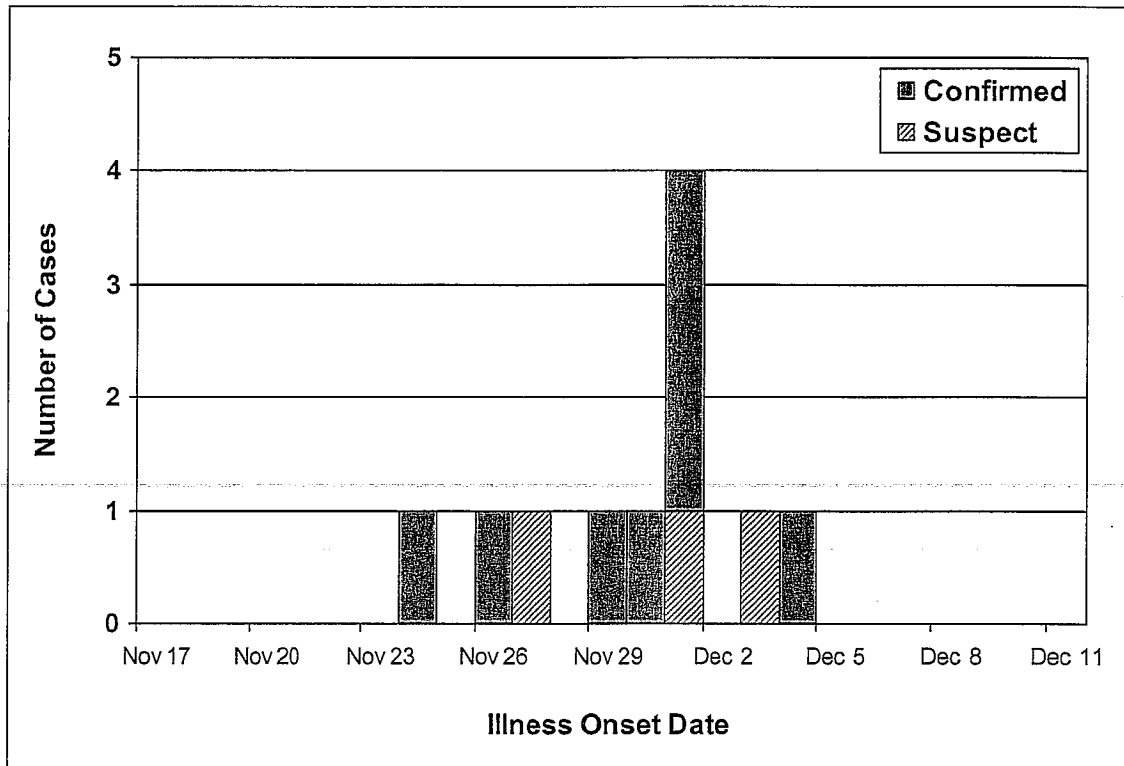
Enhanced Surveillance

In response to the December 12 email, five additional persons with confirmed *Campylobacter* infection who reported drinking Dairy A raw dairy product prior to illness onset, along with two epidemiologically-linked suspect cases, were identified. Both of the epidemiologically-linked suspect cases lived with a confirmed case-patient, and had also consumed Dairy A raw dairy product prior to onset of their diarrheal illness.

In response to the December 20 request for enhanced surveillance, 12 local health jurisdictions submitted 80 completed questionnaires for patients with confirmed *Campylobacter* infection. Illness onsets ranged from November 6 to December 31, 2007. Patient ages ranged from 7 weeks to 89 years (median 40 years); 58% were female. Most (96%) patients were symptomatic with diarrhea (94%), abdominal cramping (70%), fever (58%), vomiting (30%), and bloody diarrhea (28%). Twelve (15%) were hospitalized for a median of 2 days (range, 1 to 4 days). Of these 80 patients, 2 (2.5%) had drunk Dairy A raw milk and 2 (2.5%) had consumed unpasteurized queso fresco from non-commercial sources. There were no common exposures among the other *Campylobacter* patients, such as a single brand of poultry, seafood, or travel exposure.

In total, eight case-patients with *Campylobacter* infection and three epidemiologically linked cases who were interviewed by local health departments reported drinking commercially available raw milk or raw colostrum from Dairy A prior to illness onset; all eight case-patients drank Dairy A milk and four also consumed raw colostrum. Case-patient ages ranged from 2 years to 55 years (median 17 years); 63% were male. The confirmed case-patients were from: Fresno (2), Los Angeles (2), Orange (2), Contra Costa (1) and Placer (1). Confirmed and suspect case-patient onset dates were from November 24 to December 4, 2007 (Figure). Other than Dairy A, no other raw milk dairy was mentioned by cases. Among these eight *Campylobacter* isolates, five were confirmed *C. jejuni*; the serotypes were unknown for the other three isolates.

Figure. Illness Onset Dates of Confirmed and Suspect *Campylobacter* case-patients with raw milk exposure, November-December 2007, California (N = 11).



Environmental Inspection

None of the case-patients had any raw dairy product left over for testing. Dates of purchase among the three case-patients who were able to recall exact purchase dates were November 20, 24, and 28, 2007.

Samples of raw whole milk (code date Nov 20), raw skim milk (code date Nov 17), and raw cream (code date Nov 22) collected on November 7, 2007, for routine pathogen testing were found negative for *Campylobacter*, *E. coli* O157:H7, *Listeria*, and *Salmonella*.

The aerobic standard plate counts for CDFA samples collected in September, October, and November of raw milk for pasteurization (RFP), raw whole milk, raw skim milk, and raw cream are presented in Table 1. From September through November 2007, the aerobic standard plate counts increased for all four types of dairy products tested.

Table 1. Aerobic standard plate counts for CDFA samples of raw milk collected September-November 2007.

Collection Date	Bulk Raw Milk (Silo Tank)*	Raw Whole Milk*	Raw Skim Milk*	Raw Cream**
September 20, 2007	<2,500	Not Tested	<2,500	5,700
October 29, 2007	<2,500	<2,500	9,900	Not tested
November 7, 2007	4,800	>250,000	>250,000	100,000

*In CFU/mL; **CFU/g

Laboratory Investigation

Only one case-patient isolate was available for PFGE testing. The PFGE patterns (SmaI pattern DBRS16.0006; KpnI pattern DBRK02.0011) from this isolate are rare (comprising 1.9% and 0.4% of *C. jejuni* patterns, respectively, in the CDC database) and did not match the PFGE patterns of any of the seven control *Campylobacter* isolates. *Campylobacter* was not recovered from any raw milk products collected on the farm or from retail outlets. However, *C. jejuni* was cultured from 9 (35%) of 26 cattle fecal samples collected at Dairy A (Table 2). Approximately 50 suspect *C. jejuni* strains were isolated from the nine positive samples, and a subset was screened by sequencing methods (MOMP and MLST) prior to sending for PFGE analysis.

CDPH MDL compared the PFGE patterns of isolates from seven of the nine bovine samples with the PFGE pattern of the case-patient isolate. Four of the seven bovine isolates had indistinguishable PFGE patterns from the case-patient isolate using both primary and secondary enzymes (Table 2).

During the laboratory investigation, other potentially pathogenic thermophilic *Campylobacter* species were identified in bovine samples including *C. coli*, *C. fetus*, *C. hyointestinalis*, and *C. lari*. Additionally, *Ochrobactrum anthropi* was cultured from cattle feces, milking barn discharge water, and a retail raw milk sample (best by date 12/25/2007).

Table 2. *Campylobacter jejuni* isolated from environmental samples collected at Dairy A, December 2007.

Sample ID	Description	PFGE Match to Case-Patient Isolate
161121707M	Cattle feces	No
161121707P	Cattle feces	Yes
161121707S	Cattle feces	No
161121707AA	Cattle feces	Yes
161121707AB	Cattle feces	N/D
161121707AC	Cattle feces	No
161121707AD	Cattle feces	N/D
161121707AE	Cattle feces	Yes
161121707AH	Cattle feces	Yes

PFGE = pulsed-field gel electrophoresis after digestion with *Sma*I and *Kpn*I enzymes using the PulseNet protocol. N/D = not done.

Discussion

In December 2007, CDPH IDB was notified of two siblings with febrile gastroenteritis; one sibling was lab-confirmed with *Campylobacter* infection and the other sibling had a negative stool specimen. Both siblings had started drinking Dairy A raw milk and raw colostrum three weeks prior to illness onset. Because *Campylobacter* outbreaks have previously been associated with consumption of raw dairy products, CDPH asked local health departments to enhance surveillance to further explore the possibility that a commercially available raw dairy product was contaminated with *Campylobacter*, and to prevent additional cases if a common source were identified.

We identified eight confirmed and three suspect cases of *Campylobacter* infection who had a history of Dairy A raw dairy product exposure prior to illness onset. These confirmed and suspect cases had illness onsets within an 11-day period in late November and early December 2007.

We attempted to determine the prevalence of raw milk exposure among California residents with *Campylobacter* infection through enhanced surveillance; 2.5% of 80 patients interviewed reported raw milk exposure. This rate is similar to an observed rate of raw milk exposure in the general population of 2.8% (2002 FoodNet population survey data for California). This finding suggests that either there was no association between the campylobacteriosis cases and raw milk consumption, or that possibly a limited amount of contaminated product was available for a brief period.

We suspect that a limited amount of contaminated Dairy A product was available for a brief period for the following reasons:

- Dairy A products: Other than for Dairy A, no other brand of raw milk was mentioned by those persons with *Campylobacter* infection who were interviewed by their health departments.
- Onset timeframe: Illness onsets for confirmed and suspect campylobacteriosis cases who consumed Dairy A raw milk products were over a short timeframe (11 days). We would expect illness onset of Dairy A product users to be spread throughout November and December if there was no association between illness and Dairy A product.
- PFGE testing: *Campylobacter* isolates from four bovine fecal samples from Dairy A had PFGE patterns indistinguishable from the available human isolate when using two enzymes. *C. jejuni* populations show high genetic diversity; therefore the discriminatory power of PFGE for *C. jejuni* is high, especially when using two enzymes. Additionally, the results of two sequenced based typing methods, MLST and MOMP, were in agreement with the PFGE results.
- *O. anthropi*: *Ochrobactrum anthropi* was cultured from cattle feces, milking barn discharge water, and a retail raw milk sample. *O. anthropi* is a naturally occurring noncoliform bacterium found in soil and water that could be an indicator of potential problems with bacterial counts and milk quality (Jayarao and Wang, 1999).
- Standard plate counts: The aerobic standard plate counts for all four types of dairy products tested by CDFA increased markedly prior to the illness onset of the campylobacteriosis cases who drank raw milk products.

A more extensive epidemiologic investigation into *Campylobacter* infections during this time would be necessary to determine if there is a statistically significant association with raw milk exposure. However, further investigation of this potential association with Dairy A raw dairy product and *Campylobacter* infection is limited by the lack of ongoing cases, only one raw dairy product associated human isolate, and no resources to continue to conduct PFGE or interview all *Campylobacter* cases.

There were several challenges with this investigation:

- The majority of local health jurisdictions do not routinely do follow up investigations on their *Campylobacter* cases as it is the most prevalent enteric pathogen, and is less commonly associated with clusters and outbreaks;
- Only 12 of 61 local health jurisdictions submitted information about their *Campylobacter* cases during the December time period;
- *Campylobacter* isolates are not forwarded to Public Health Laboratories and are kept very briefly by clinical laboratories, hence, only one human isolate remained viable for subtyping by PFGE;
- PFGE of *Campylobacter* is not routinely conducted; therefore, clusters are not identified through PFGE;
- Only one case-patient isolate was available for testing. Although this isolate had PFGE patterns that were rare and matched those of isolates from cattle at Dairy

A, the possibility that other strains of *Campylobacter* could have been isolated from other case-patients cannot be excluded.

Since none of the persons with *Campylobacter* infection who became ill after December 4, 2007 had any consistently common exposures, we concluded that this risk was not ongoing.

Recommendations

- Maintain surveillance for additional cases of *Campylobacter* associated with raw dairy products
- Continue public health education efforts regarding the risk of infection with consuming raw dairy products

References

1. Centers for Disease Control (CDC). *Campylobacter* outbreak associated with raw milk provided on a dairy tour--California. *MMWR Morb Mortal Wkly Rep*. 1986 May 16;35(19):311-2.
2. Doyle MP, Roman DJ. Prevalence and survival of *Campylobacter jejuni* in unpasteurized milk. *Appl Environ Microbiol*. 1982 Nov;44(5):1154-8.
3. Jayarao BM and Wang L. A study on the prevalence of gram negative bacteria in bulk tank milk. *J Dairy Sci*. 1999 Jul;89(7):2620-24.
4. Klein BS, Vergeront JM, Blaser MJ, Edmonds P, Brenner DJ, Janssen D, Davis JP. *Campylobacter* infection associated with raw milk. An outbreak of gastroenteritis due to *Campylobacter jejuni* and thermotolerant *Campylobacter fetus* subsp *fetus*. *JAMA*. 1986 Jan 17;255(3):361-4.
5. Kornblatt AN, Barrett T, Morris GK, Tosh FE. Epidemiologic and laboratory investigation of an outbreak of *Campylobacter* enteritis associated with raw milk. *Am J Epidemiol*. 1985 Nov;122(5):884-9.
6. Korlath JA, Osterholm MT, Judy LA, Forfang JC, Robinson RA. A point-source outbreak of campylobacteriosis associated with consumption of raw milk. *J Infect Dis*. 1985 Sep;152(3):592-6.
7. Peterson MC. *Campylobacter jejuni* enteritis associated with consumption of raw milk. *J Environ Health*. 2003 May;65(9):20-1, 24, 26.
8. Potter ME, Blaser MJ, Sikes RK, Kaufmann AF, Wells JG. Human *Campylobacter* infection associated with certified raw milk. *Am J Epidemiol*. 1983 Apr;117(4):475-83.
9. Wood RC, MacDonald KL, Osterholm MT. *Campylobacter* enteritis outbreaks associated with drinking raw milk during youth activities: A 10-year review of outbreaks in the United States. *JAMA*. 1992 Dec 9;268(22):3228-30.