CA EPI 11-03: Cluster of *Escherichia coli* O157:H7 Infections Associated With Raw Milk Consumption
March 12, 2012

Summary

In November 2011, a cluster of five young children with *Escherichia coli* (*E. coli*) O157:H7 infection with matching pulse-field gel electrophoresis (PFGE) patterns was identified. Illness onsets were from August 25 to October 25, 2011. All five children reported drinking commercially available raw (unpasteurized) milk from a single dairy (Organic Pastures) and had no other common exposures. Statistical analysis of case-patients' exposures with a comparison group of *E. coli* O157:H7 patients with non-cluster PFGE patterns indicated a strong association with raw milk. The epidemiological findings led to a quarantine and recall of all Organic Pastures products except cheese aged more than 60 days, and investigations by the California Department of Public Health (CDPH) Food and Drug Branch (FDB) and the California Department of Food and Agriculture (CDFA). Environmental samples collected at Organic Pastures yielded *E. coli* O157:H7 isolates that had PFGE patterns indistinguishable from the patient isolates. Organic Pastures raw milk consumed by the case-patients was likely contaminated with this strain of *E. coli* O157:H7, resulting in their illnesses.

Introduction

On November 9, 2011, the CDPH Microbial Diseases Laboratory (MDL) notified the CDPH Infectious Diseases Branch (IDB) of five shiga toxin-producing *E. coli* (STEC) O157:H7 isolates with matching PFGE pattern combination (Xbal EXHX01.2294 / Bln EXHA26.0071). This PFGE pattern is uncommon in California and had not been seen since January 2010. The pattern had been seen in other states in the preceding six months and had been associated with a petting zoo outbreak in Washington State in June 2011.

The case-patients were residents of Contra Costa (2), Kings, Sacramento, and San Diego counties with illness onset dates from August 25 to October 25, 2011. Three were hospitalized with hemolytic uremic syndrome (HUS) and required peritoneal dialysis. Initial review of the standardized case report forms indicated that all patients reported drinking Organic Pastures raw whole milk in the week prior to illness onset.

IDB implemented enhanced surveillance to identify additional cases and conducted an epidemiological analysis to determine if the illnesses were associated with exposure to Organic Pastures raw milk or another source. CDPH FDB and CDFA were immediately notified of the cluster.

Methods

Epidemiologic Investigation

All STEC and HUS cases are reportable in California and all case-patients are interviewed by local health jurisdictions (LHJs) using a standardized STEC case report form. In addition, all STEC isolates are requested to be forwarded to public health laboratories (PHLs) for PFGE analysis and the results are subsequently uploaded to the national PulseNet database.

On November 9, 2011, IDB alerted all California LHJ Communicable Disease controllers of the cluster investigation. LHJs were requested to promptly interview all patients with STEC infection or HUS identified through routine surveillance. LHJs were also requested to notify IDB immediately if a STEC or HUS patient reported raw milk exposure prior to illness onset, as well as to collect any remaining raw milk products for laboratory testing.

A case was defined as laboratory-confirmed *E. coli* O157:H7 infection with cluster-strain PFGE pattern (Xbal EXHX01.2294 / Bln EXHA26.0071) in a California resident with illness onset on or after July 1, 2011.

Parents of the case-patients were interviewed using a supplemental questionnaire (Attachment A) to confirm the reported exposures and to obtain additional details about the raw milk product(s), including the date and location of purchase. The supplemental questionnaire further explored known *E. coli* O157:H7 risk factors and exposures, including exposure to farms or livestock. Because one family reported using several health foods and health food supplements, questions about these exposures were also included.

A case-case analysis was conducted comparing the frequency of risk factors and exposures reported on the standardized CDPH STEC report form among case-patients and non-cluster strain *E. coli* O157:H7 patients 10 years of age or younger with illness onset dates on or after July 1, 2011. Univariate odds ratios were calculated using Chi-square test for each risk factor and two-sided p-values were calculated using Fisher's exact test. Statistical significance was set at $P \leq 0.05$.

The frequency of food exposures reported among the cases were also compared to that of a comparable general population from the 2006-2007 Foodborne Diseases Active Surveillance Network (FoodNet) Population Survey Atlas of Exposures (http://www.cdc.gov/foodnet/surveys/FoodNetExposureAtlas0607_508.pdf) for California, applying a cumulative binomial probability calculation.

MDL reviewed the historic database of all California *E. coli* O157:H7 PFGE patterns to determine whether the current illness cluster PFGE pattern had been seen previously in California.

Laboratory Investigation

All five patients' *E. coli* O157:H7 stool isolates were PFGE tested by CDPH MDL. Additionally, the isolates were forwarded to the U.S. Centers for Disease Control and Prevention (CDC) for multilocus variable number tandem repeat analysis (MLVA) testing.

One family had made kefir (similar to drinkable yogurt) from raw whole milk (expiration date October 12, 2011), and used the whey from the kefir to make pickles. Leftover kefir and pickles were both available, and tested for the presence of enteric pathogens by the Contra Costa County PHL. Contra Costa County PHL also tested a retail sample of Organic Pastures whole milk (expiration date November 9, 2011), purchased at the same store as the family. The family's leftover samples were also sent to the United States Department of Agriculture (USDA) Agricultural Research Services (ARS) laboratory in Albany, California for laboratory testing. No other left-over samples from other case-patients were available for testing.

Environmental Investigations

Inspection of Organic Pastures and collection and testing of environmental and product samples were carried out by CDFA and the CDPH FDB. Descriptions of those investigations are reported separately.

Results

Epidemiologic Investigation

No additional culture-confirmed cases were identified through enhanced surveillance. The median age of the five case-patients was 4 years old (range 1-5 years), all were male, and two were siblings. Three developed HUS and required peritoneal dialysis; there were no deaths. On November 28th, IDB was notified of a 17-month-old Mendocino County patient hospitalized with post-diarrheal HUS, requiring peritoneal dialysis. The suspect case's illness onset was November 11 and the child had reportedly consumed raw butter and raw cheese from Organic Pastures in the week prior to illness. Samples of the child's stool, an empty butter tub, and leftover cheese provided by the family were tested by MDL and FDB. However, as none of the tested samples were positive for shiga toxin presence or yielded STEC, this patient was not included as part of the cluster.

The CDC PulseNet database identified five non-California (Massachusetts, Tennessee [2], Oregon, and Washington) cases of *E. coli* O157:H7 with PFGE pattern matching the 2011 California cluster in the previous four months. Specimen collection dates for these patients ranged from July 24 to October 29, 2011. The non-California patients did not travel to California, were demographically different, and did not report exposures common to the California case-patients. These non-California patients, therefore, were also not considered as part of the cluster.

California pre-2011 cases

Prior to 2011, there have been five California cases of *E. coli* O157:H7 with PFGE pattern matching the current illness cluster. Two cases occurred in 2009 and three in 2010. None of these patients reported raw milk exposure at the time of the initial interviews. The parents of two of these families were reached by telephone and they again denied exposure to raw milk prior to their child's illness onset.

Case-case comparison

A comparison group of forty-seven *E. coli* O157:H7 patients who were 10 years of age or younger with non-cluster PFGE pattern with illness onset dates during July 1 – November 11, 2011 were identified in CDPH routine surveillance reports. Drinking raw milk was strongly associated with the cluster cases compared to the non-cluster *E. coli* O157:H7 patients (odds ratio undefined, $P < 0.001$). No other exposures were significantly associated with the cluster pattern **(Table 1)**.

Table 1. Comparison of risk factors reported for case-patients and non-cluster *E. coli* O157:H7 patients ≤10 years of age, July 1-November 11, 2011.

Risk factor	Case-patients (n=5)	Non-cluster patients (n=47)	Odds Ratio	P-value*
Any ground beef	3	34	0.53	0.61
Undercooked ground beef	1	5	1.9	0.50
Untreated water	0	9	Undefined	0.57
Unpasteurized juice	0	2	Undefined	1.0
Sprouts	0	1	Undefined	1.0
Pre-packaged spinach	1	1	9	0.23
Petting zoo / livestock	0	14	Undefined	0.31
Any raw milk/ raw milk products	5	0	Undefined	0.00000043

*Two-sided Fisher's Exact Test

Cumulative binomial probability

Compared to the 3 percent California population estimate of raw milk consumption published by FoodNet, the calculated cumulative binomial probability of finding raw milk exposure among all five of the cases was 2.4×10^{-8}, which is less than one in 10 million.

Laboratory Investigations

Stool isolates of all five cases underwent PFGE testing at CDPH MDL. The PFGE pattern combination (Xbal EXHX01.2294 / Bln EXHA26.0071) among the case isolates were indistinguishable. According to CDC, this pattern ranks 26 out of 7066 in the CDC

PulseNet database and had been seen 48 times nationally between August 2007 and November 2011.

MLVA testing of the isolates by CDC revealed indistinguishable patterns among the five California case-patients. Isolates from two of the five non-California patients were available for MLVA testing by CDC, and they revealed MLVA patterns that were distinct from the 2011 California cluster.

The clinical isolates from the pre-2011 California cases were also forwarded to CDC for MLVA testing and four have been completed. Three of the historical California isolates (two of which match each other by MLVA) are not closely related to the 2011 cluster MLVA pattern. One isolate collected in 2009 has a MLVA pattern that is highly related to the 2011 cluster. However, the case report from this case did not indicate raw milk exposure.

Contra Costa County PHL's testing of one family's leftover kefir and pickles made from raw whole milk and whey did not yield STEC. The results of sample testing by the USDA ARS laboratory were also negative.

<u>Environmental Investigation</u>

Detailed results of the environmental investigation and testing at Dairy A are reported separately. Ten of the samples collected from the calf area were positive for *E. coli* O157:H7 (1 swab, 3 soil, 1 water, and 5 fecal), of which two of the isolates (1 fecal and 1 soil) had a PFGE pattern indistinguishable from the cluster strain. These two isolates were submitted to the CDC for MLVA analysis and the isolate from the soil was found to be highly related to the human cluster MLVA pattern and identical to one of the historical California human isolates from 2009 (results are still pending for the fecal isolate).

Discussion

In the fall of 2011, we investigated an outbreak of *E. coli* O157:H7 with an unusual PFGE pattern not seen in California since January 2010. Organic Pastures raw milk consumption was implicated as the source of the outbreak based on the following epidemiological and laboratory data:
- All five patients had identical strains by PFGE and MLVA molecular testing;
- All five patients had consumed raw milk from Organic Pastures and had no other exposures in common;
- No other risk factors besides raw milk consumption were statistically significant in a case-case analysis comparing case-patients with non-cluster *E. coli* O157:H7 patients ≤10 years old during the cluster period;
- Given that only 3% of the California general population reported consuming raw milk, the probability of all five case-patients consuming raw milk is less than one in 10 million;

- Matching PFGE strains of *E. coli* O157:H7 were found in the Organic Pastures environment, and the MLVA pattern for one of the environmental strains was highly related to the cluster strain.

Raw milk and raw milk products are commercially available in California, and have previously been associated with outbreaks or clusters of bacterial infections, including *E. coli* O157:H7, *Campylobacter, Listeria* and *Salmonella*. In 2006, IDB conducted an epidemiological investigation involving five young children with matching *E. coli* O157:H7 PFGE pattern from four California counties and concluded that consuming raw milk or raw colostrum from Organic Pastures was likely the source of the illnesses. Organic Pastures has also had previous recalls of products due to detections of *Campylobacter* and *Listeria monocytogenes*.

Partly as a result of the 2006 *E. coli* O157:H7 outbreak associated with raw milk, California enacted regulations in 2008 that set the upper limit of coliforms/mL of raw milk to 10. However, given that illnesses associated with commercial raw milk continue to occur, continued efforts are needed to educate consumers about the potential risks of infection and severe illnesses, including kidney failure, from raw milk consumption.

Our investigation is subject to limitations. Although we initiated enhanced surveillance by notifying all Communicable Disease controllers in California, additional illnesses associated with raw milk consumption might have occurred that were not diagnosed and/or reported. Additionally, there were no leftover samples of raw milk from lots consumed by the case-patients available for testing due to the short shelf-life of dairy products. Finally, the tested samples of leftover milk products did not yield STEC. Nonetheless, data from the epidemiologic, laboratory, and environmental investigations strongly implicate Organic Pastures raw milk as the source of the illnesses.

Recommendations

- Maintain surveillance for *E. coli* O157:H7 cases. Improve the timeliness of cluster identification by monitoring STEC and HUS case reports that indicate raw milk exposure to prioritize PFGE testing and follow-up.
- Continue public education efforts regarding the risks of raw milk and raw milk product consumption, especially the greater risk of severe bacterial diseases among children, pregnant women, the elderly, and persons with weakened immune systems.

Appendix A: Supplemental Questionnaire

CASE ID: _____

E. coli O157:H7 Supplemental Questionnaire *Version 1111*

Patient Name: _____ Age:_____ Local Health Jurisdiction:_____

Phone(s): home (___)_____ cell (___)_____ other (___)_____

Date of onset:_____/____/_____ Culture confirmed?_____ PFGE?_____

Interviewer:_____ Date of interview:_____

GENERAL INFORMATION

1. Do you have any food allergies, or foods you simply would not eat? Y   N   DK
    If Yes, Specify:_____

2. In the TWO WEEKS before illness onset, did you have close contact with someone with diarrheal illness? Y N DK
    a. *If Yes:* Who? _____   b. Nature of contact:_____
    c. Date of onset:_____   d. Phone number:_____

3. In the TWO weeks before illness onset, did you travel outside your county of residence? Y   N   DK
    a. *If YES:* Where?_____   Dates of travel:_____

4. In the TWO weeks before illness onet, did you have any exposure to livestock or petting zoos? Y   N   DK
    a. *If YES:* Describe_____   Dates of exposure_____

5. Do you know of anyone else with a simliar illness? Y N DK
    a. *If Yes:* Who? _____   b. Nature of contact:_____
    c. Date of onset:_____   d. Phone number:_____

6. In the TWO weeks before illness onset, did you use health food items or supplements (e.g., vitamins, minerals, nutritional yeast, flax seeds, etc)? Y   N   DK
    a. If Yes, what? _____   b. Brand name_____
    c. Location of purchase _____   d. Date(s) of use _____

SUSPECT FOODS

In the TWO WEEKS before becoming ill, did you consume any of the following? *If yes to any, complete additional information on table.*

Yes	No	Exposure	Raw/ under-cooked? Yes	No	Description/Brand	Where purchased or obtained
		Any preformed hamburger patties at home				
		Any other ground beef at home				
		Hamburger outside of home (e.g., BBQ, restaurant)				
		Cheese				
		Pre-packaged salads				
		Pre-packaged spinach				
		Sprouts				
		Any raw milk or raw milk products including colostrum**				

**If answers yes to this question, please fill out Page 2 (Milk supplement)

CASE ID:

RAW/UNPASTEURIZED MILK & MILK PRODUCT FOLLOW UP QUESTIONS

In the TWO weeks before your symptoms started, what types of raw/unpasteurized milk or milk products did you consume? Please let us know even if you think you may have just had a small taste or sample. *Include product details listed in the table below.*

Type of raw milk product (milk, cheese, yogurt, butter, colostrum, etc.)	Product specifics (% fat, type of cheese, etc.)	How often consumed	Brand Name (in CA, Organic Pastures & Claraville	Lot no. or code	Expiration date	Purchase date	Do you have a receipt?		Where product obtained (dairy, store, restaurant, friend's home, etc.)	Any product left?		Can we take sample for lab testing?*	
							Yes	No		Yes	No	Yes	No
1.		☐ Every day ☐ More than once a week but not every day ☐ Just once											
2.		☐ Every day ☐ More than once a week but not every day ☐ Just once											
3.		☐ Every day ☐ More than once a week but not every day ☐ Just once											
4.		☐ Every day ☐ More than once a week but not every day ☐ Just once											
5.		☐ Every day ☐ More than once a week but not every day ☐ Just once											

*If willing to give some leftover product for testing, ask them to keep it refrigerated/on ice and make arrangements to have it picked up.

Interviewer name: _____   Date: _____ / _____ / _____

Please FAX completed questionnaires to Joyanna Wendt at 510.620.3425. Thank you.