MAJOR ARTICLE

Outbreak of *Escherichia coli* O157 Associated with Raw Milk, Connecticut, 2008

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(See the editorial commentary by Jay-Russell, on pages XXX–XXX.)

Background. In Connecticut, despite hazards of raw milk consumption, attempts to ban raw milk sales have been unsuccessful. In July 2008, 2 children experienced *Escherichia coli* O157–associated hemolytic uremic syndrome (HUS) after consuming raw milk purchased at a retail market and a farm (farm X). We investigated to determine the outbreak source and control measures.

Methods. Confirmed cases were HUS diagnosis or *E. coli* O157:NM infections with isolates matching outbreak strains among patients during June to July 2008. Probable cases were diarrheal illness among farm X customers during the same period. We conducted case-control studies to determine the source of *E. coli* O157 exposure and assess for dose-response relation between illness and frequency of raw milk consumption. Farm X dairy practices were evaluated; stool specimens of humans and animals were cultured for *E. coli* O157. Staff time and laboratory and medical costs were calculated.

Results. We identified 14 cases (7 confirmed). Five (36%) case patients required hospitalization; 3 (21%) experienced HUS. No deaths were reported. Raw milk consumption was associated with illness ($P = .008$); a dose-response relation was demonstrated ($P = .01$). Dairy practices reflected industry standards. *E. coli* O157:NM outbreak strains were isolated from stool specimens of 6 case patients and 1 milking cow. The total estimated outbreak cost was $413,402.

Conclusions. Farm X's raw milk was the outbreak source despite no violations of current raw milk regulatory standards. This outbreak resulted in substantial costs and proposed legislation to prohibit nonfarm retail sale, strengthen advisory labels, and increase raw milk testing for pathogens.

Among Shiga toxin–producing *Escherichia coli*, *E. coli* O157:NM strains have emerged as important human pathogens producing a similar clinical spectrum of disease as *E. coli* O157:H7 [1, 2]. Illness ranges in severity from diarrhea to hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenia purpura (TTP) and even death. Children and older persons are particularly vulnerable to severe illness [3, 4]. Among children with HUS, the mortality rate is 3%–5% [5, 6]. Because cattle are a major reservoir for Shiga toxin–producing *E. coli* [7], undercooked ground beef is the most common cause of *E. coli* O157 infections [8, 9]. However, severe illnesses attributed to raw milk have recently emerged, especially among children [10, 11].

Although raw milk is consumed by an estimated 1%–3% of the US population, raw milk or raw milk cheese is responsible for ~70% of reported dairy outbreaks [12, 13]. The US Food and Drug Administration banned interstate distribution of unpasteurized dairy products in 1987 [14]. As of 2008, however, a total of 29 states still permit intrastate sale of these products; 13 of these states also allow raw milk sales in retail markets [15]. In Connecticut, raw milk sales are legal on farms and in retail markets. Despite the well-documented risk for enteric infection [10, 16–19], past attempts by state officials to ban raw milk sales in Connecticut have been unsuccessful.

On 16 July 2008, the Connecticut Department of

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Public Health (CT DPH), through routine surveillance, identified 2 unrelated children who had experienced HUS 1 week after consuming raw milk purchased from a farm (farm X). *E. coli* O157:NM was isolated from the stool specimen of 1 of the children. In this article, we describe our investigation and report an estimated outbreak cost and proposed legislation to minimize future occurrences of raw milk–associated outbreaks in Connecticut.

PATIENTS AND METHODS

Case finding. A confirmed case was defined as either *E. coli* O157:NM infection with an isolate that was indistinguishable from the outbreak pulsed-field gel electrophoresis (PFGE) patterns in a Connecticut resident during June to July 2008 or an HUS diagnosis in a Connecticut resident during the same period. A probable case was defined as diarrheal illness ($\geq$2 loose stools per day for $\geq$2 days) in a farm X customer during the same period.

Because HUS and *E. coli* O157 infections are reportable conditions in Connecticut, we reviewed surveillance and laboratory reports for cases. In addition, we contacted all Connecticut infectious disease physicians, emergency departments, and laboratories to identify any new cases that had not been reported yet. We also assessed for diarrheal illness among farm X customers and their household members by contacting them by telephone from a customer list provided by the farm.

Community case-control study. Confirmed case patients were matched to 2 control subjects each on the basis of neighborhood and age group (<18 years or $\geq$18 years). Control subjects were identified through an online telephone directory (http://www.whitepages.com) and did not have diarrheal illness since June 1. All confirmed case patients and matched control subjects (or parents of children aged <18 years) were asked about exposures to well-documented sources of *E. coli* O157 during the week preceding illness onset of case patients.

Household case-control study. In this unmatched study, we assessed for a dose-response relation between frequency of raw milk consumption and illness among all confirmed and probable case patients and their household members. Control subjects were well household members without diarrheal illness during June to July 2008. We determined the frequency of raw milk consumption by all household members during the 1 week before illness onset in case patients.

Environmental investigation. We visited farm X to assess its milking procedures, processing and packaging of raw and pasteurized milk, and cleaning and sanitization of equipment. We asked workers about gastrointestinal illnesses to identify cases and to determine whether a worker could have been a source of transmission. We also reviewed results of on-site raw milk testing conducted weekly by farm X and monthly by the Connecticut Department of Agriculture (CT DAG) for elevated levels of coliform bacteria to indicate possible fecal contamination. Environmental (ie, milk contact surfaces, animal feeding areas, and mud), milk, and bovine fecal specimens were collected for testing.

Laboratory analysis. The CT DPH laboratory tested all collected samples for *E. coli* O157, simultaneously using molecular screening methods by polymerase chain reaction and conventional microbiologic culture methods. All isolates were tested for sorbitol fermentation and subtyped by PFGE using restriction endonucleases [20]. *E. coli* isolates from human stool were characterized at the Centers for Disease Control and Prevention by toxin profiling and multilocus variable-number tandem repeat analysis [21, 22]. One human serum sample was tested at the Centers for Disease Control and Prevention for *E. coli* O157 antibodies.

Estimating outbreak cost. Using 2008 US dollars, we estimated the cost of the investigation and medical expenses incurred from hospitalizations of case patients. Personnel time was converted to cost by multiplying a person's gross wage by time spent on the investigation. Counts and unitary costs were obtained for specimens collected, laboratory reagents used, and miles traveled. Fringe benefits and indirect costs were included. Estimates of total cost or charges of hospitalization were provided by hospitals.

Statistical analysis. Descriptive statistics were used to summarize demographic and clinical information. We performed exact conditional logistic regression to determine the associations between exposures and illness. Matched odds ratios and 95% confidence intervals were calculated; P values of <.05 were considered to be statistically significant. We assessed for a dose-response relation between frequency of raw milk consumption and illness by using χ^2 test for trend. SAS statistical software, version 9.1.3 (SAS Institute), was used for analysis.

RESULTS

Characteristics of cases. We identified 14 cases; 7 (50%) were confirmed. Of these 7 cases, 6 (86%) met the case definition by a laboratory diagnosis and 1 (14%) by an HUS diagnosis. Among 44 persons of 16 farm X households, 7 persons were identified who met the probable case definition. Median age of the 14 confirmed and probable case patients was 5 years (range, 1–81 years); 10 (71%) were children aged <18 years.

Onsets of illness occurred from 1 June through July 28 (Figure 1). Among 5 hospitalized case patients, 1 was an adult (20%) with TTP who required plasmapheresis and 4 (80%) were children, 3 of whom had HUS and required dialysis (Table 1). None of the case patients had received antimicrobials during the 3 weeks before illness. However, 1 case patient had received antimicrobials after illness onset and was hospitalized within 1 week of receiving antibiotics with diagnosis of TTP. The other 3 case patients who had HUS were diagnosed shortly after

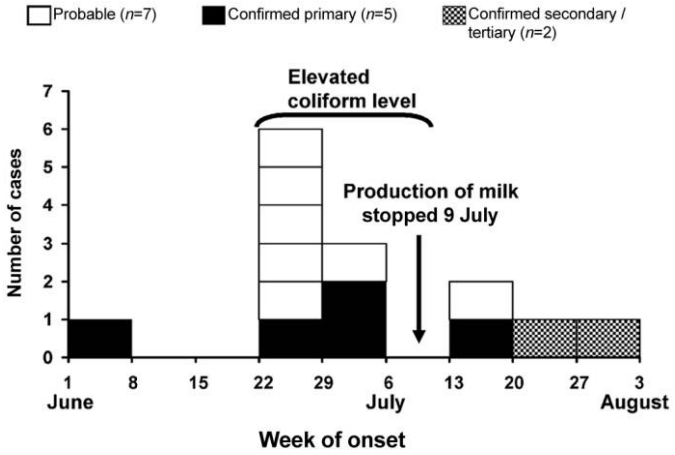

Figure 1. *Escherichia coli* O157 cases in Connecticut, June–July 2008 ($n = 14$).

presentation of their illness and were not prescribed antibiotics during illness. No deaths were reported.

Community case-control study. At the time we conducted the community case-control study, we had identified 5 confirmed cases. Only raw milk consumption was significantly associated with developing illness ($P = .008$). The matched odds ratio for raw milk exposure was undefined because all 5 case patients had consumed farm X raw milk, whereas none of the 10 control subjects had raw milk exposure (Table 2). Three (60%) of the 5 case patients had purchased the milk in a retail market. All 5 confirmed case patients (or their parents) reported having prior knowledge that raw milk can contain disease-causing bacteria.

Household case-control study. Among 33 members of 9 households in which the 5 confirmed primary case patients and 7 probable case patients lived, 12 case patients and 21 well household members were identified. Overall, 23 persons had consumed raw milk, 11 (46%) of whom subsequently experienced illness. A significant trend of more frequent raw milk consumption was demonstrated among case patients, compared with well household members ($P = .01$) (Table 3).

Secondary and tertiary transmission. After the case-control studies were conducted, 2 additional confirmed case patients were identified, neither of whom had exposure to raw milk or to other common sources of *E. coli* O157. One of the 2 was a sibling aged 12 months of a primary case patient who had consumed farm X raw milk purchased at a retail market. The other was a neighbor aged 2 years who had frequent close contact with the sibling (Figure 1). The PFGE pattern of *E. coli* O157:NM isolated from the stool of these 2 patients was indistinguishable from that of the primary case patient. Both the sibling and the neighbor were thought to have experienced disease from secondary and tertiary transmission, respectively.

Environmental investigation. Farm X produced and sold raw and pasteurized milk and other pasteurized dairy products on the premises. Both raw and pasteurized milk were also distributed to 4 nearby retail markets that sold natural and organic foods.

The milk production area consisted of a stanchion barn with an attached storage room and a processing plant located in a separate building with a room for retail sales. Milking of 28 Jersey cows occurred in the barn via a vacuum pipeline system, which drained all milk into a bulk tank in the storage room. Twice weekly, raw milk was pumped from the bulk tank through a rubber hose into a refrigerated portable tank and transported ~150 ft (~45.7 m) to the processing plant. One-half of the transported milk was pasteurized, and the remaining milk was packaged in bottles with "Raw Jersey Milk" printed on the cap. A consumer advisory label indicating that unpasteurized milk can contain harmful bacteria was not affixed to the bottles or printed on attached hangtags [23]; however, signage with the

Table 1. Demographic and Clinical Characteristics of Case Patients in an Outbreak of *Escherichia coli* O157 Infection in Connecticut, 2008 ($n = 14$).

Characteristic	Case patients
Age, median years (range)	5 (1–81)
Female	8 (57)
Diarrhea	14 (100)
Bloody diarrhea	6 (43)
Vomiting	5 (36)
Fever	4 (29)
Hospitalization	5 (36)
Hospital stay, median days (range)	16 (1–33)
HUS or TTP	4 (29)
Dialysis or plasmapheresis, proportion (%)	4/4 (100)
Death	0 (0)

NOTE. Data are no. (%) of patients, unless otherwise indicated. HUS, hemolytic uremic syndrome; TTP, thrombotic thrombocytopenic purpura.

Table 2. Community-Matched Case-Control Study: Source of Exposure to *Escherichia coli* O157

	Stratum 1			Stratum 2			Stratum 3			Stratum 4			Stratum 5				
E. coli O157 exposure	C 1	Con 1A	Con 1B	C 2	Con 2A	Con 2B	C 3	Con 3A	Con 3B	C 4	Con 4A	Con 4B	C 5	Con 5A	Con 5B	mOR (95% CI)	*P*
Raw milk	Y	N	N	Y	N	N	Y	N	N	Y	N	N	Y	N	N	…	.008
Tomatoes	N	N	Y	N	Y	…	Y	Y	…	Y	Y	Y	Y	N	N	1.20 (0.08–18.10)	>.99
Whole head lettuce	Y	N	N	N	Y	…	Y	N	N	…	Y	N	N	N	N	3.24 (0.16–196.59)	.67
Raw cheese	N	N	N	N	N	N	Y	N	N	Y	N	N	N	N	N	…	.22
Spinach	…	N	Y	N	N	…	…	N	N	Y	Y	N	N	N	N	…	>.99
Ground beef	N	Y	Y	N	Y	…	Y	Y	Y	N	Y	N	N	Y	Y	…	.44
Bagged lettuce	N	N	Y	N	Y	…	N	Y	N	Y	Y	Y	N	N	N	…	>.99
Swimming	N	Y	Y	Y	Y	…	N	Y	N	Y	Y	Y	Y	Y	Y	…	.44
Visit farm	N	N	N	Y	N	…	Y	N	N	N	N	N	N	N	N	…	.33
Contact farm animals	N	N	N	N	N	…	Y	…	N	N	N	N	N	N	N	…	>.99

NOTE. Exposure data for each case patient and matched control subjects (designated as A and B, respectively) by strata are shown. C, case patient; CI, confidence interval; Con, control subject; mOR, matched odds ratio; N, no exposure was reported; Y, exposure was reported. Dash denotes missing or undefined variable.

advisory was posted at the point of sale. Occasionally, raw milk was directly bottled from the bulk tank in the storage room by manually attaching one end of a steel tube to the bulk tank outlet and the other end to a bottle. All processing equipment was cleaned after use and sanitized just before the next use.

Overall, milking and disinfection procedures did not reveal substantial regulatory violations. However, specific practices and observations of concern included manual bottling of raw milk directly from the bulk tank, lack of hand soap and malfunctioning hot water knob at the hand sink in the storage room where manual bottling occasionally occurred, failure to cap valves, and the presence of a biofilm inside the portable tank. In addition, weekly raw milk testing conducted voluntarily by farm X using a private laboratory revealed elevated levels of coliform bacteria (>50 CFU/mL) in 3 separate samples that were collected during the last week of June and the first 2 weeks of July. As a result, farm X voluntarily suspended its raw milk sales on 9 July. Four case patients experienced illness after the suspension of sales; 2 of these patients had consumed raw milk produced before the suspension, and the other 2 were infected from secondary and tertiary transmission, respectively (Figure 1). Raw milk samples collected monthly by CT DAG before this period complied with regulatory standards. Because farm X raw milk was no longer sold by the time of this investigation and because raw milk has a short shelf-life, the CT DAG did not issue a product recall.

Farm X had 11 workers at the time of this investigation. One worker reported diarrheal illness on 11 July, but a stool sample collected on 20 July tested negative for *E. coli* O157 by polymerase chain reaction and culture.

Laboratory analysis. Non–sorbitol-fermenting *E. coli* O157:NM that produced Shiga toxin were isolated from the stool of 6 of the 7 confirmed case patients. Five (83%) yielded an indistinguishable PFGE pattern (pattern A), and 1 (17%) had a PFGE pattern that differed from pattern A by 1 band. However, testing performed on this 1 isolate and 3 others with pattern A indicated all 4 shared an indistinguishable multilocus variable-number tandem repeat analysis pattern and produced Shiga toxin 2. One clinically confirmed case patient without *E. coli* O157 isolated from stool had a serum sample with an elevated antibody titer to *E. coli* O157.

Environmental specimens that were tested consisted of 97 milk samples, 39 environmental samples, and fecal samples from 34 farm animals, including the 28 dairy cows. Of the 170 specimens tested, *E. coli* O157:NM with PFGE pattern A was isolated from a bovine fecal specimen.

Outbreak cost. The total estimated outbreak cost during an approximate 3-month period was $413,402 (Table 4). The average medical cost for a hospitalized case patient was $72,904; notably, 1 patient with TTP contributed $209,571 to the total medical expense. The average cost per case patient incurred by investigative and response activities was $3491.

Table 3. Household Case-Control Study: Dose-Response Relation between Illness and Frequency of Raw Milk Consumption

	No. (%) of subjects		
Raw milk consumed, times per week	Case patients (= 12)	Control subjects (= 21)	*P* value for trend
0	1 (8)	9 (43)	
1–3	2 (17)	6 (29)	
4–7	4 (33)	3 (14)	
⩾8	5 (42)	3 (14)	.01

Table 4. Estimated Public Health and Direct Hospitalization Costs of an Outbreak of *Escherichia coli* O157 Infection in Connecticut, 2008

Outbreak	No. of personnel hours[a]	Cost, $US[b]
Investigative and response activities		
Active case finding	20.25	1285.96
Patient interviews	60.75	4051.02
Data entry and analyses	28.75	2252.84
Assessment of dairy practice	93.5	6081.39
Environmental and milk sample collection	65.5	3799.19
Implementing control measures	49.5	4098.62
Communication internally and with public	119.25	10,277.35
Laboratory		
Testing human/bovine specimen	155	5800.00
Testing farm/milk specimens	55	2070.00
Laboratory materials/reagents	…	8700.00
Transportation (794 miles)[c]	…	464.50
Total	647.5	48,880.87
Medical expense[d]		
4 Pediatric patients	…	154,950.00
1 Adult patient[e]	…	209,571.25
Total	…	364,521.25
Outbreak total	647.5	413,402.12

NOTE. Data are for an ~3-month period: June through August.

[a] Personnel hours included business and nonbusiness hours (ie, during weekends or evenings) allocated to the activity. Ellipses denote not applicable.

[b] Fringe benefits and indirect costs were included.

[c] Mileage cost was calculated as $0.585 per mile.

[d] Outpatient medical costs were not included.

[e] Medical expense reflects amount charged instead of actual cost.

DISCUSSION

Several findings from this investigation indicate that consumption of farm X raw milk caused this *E. coli* O157:NM outbreak. Raw milk was the only exposure source associated with illness; all confirmed primary case patients and none of the control subjects were exposed to farm X raw milk. In addition, a dose-response relation between raw milk consumption and illness was demonstrated. Furthermore, *E. coli* O157:NM was isolated from a fecal specimen of a farm X dairy cow that matched the predominant PFGE outbreak strain.

Although the exact mechanism of raw milk contamination cannot be determined, we suspect that fecal contamination with *E. coli* O157 from at least 1 asymptomatic cow occurred during milking or the handling of milk. Notably, contamination occurred despite acceptable milking and sanitation procedures, according to regulatory standards. As demonstrated in this and previous outbreaks, careful dairy practices can only minimize, but not completely eliminate, the risk for raw milk contamination [16, 17]. Given the anatomical location of a cow's udder and the presence of bovine feces in the barn where milking occurs, numerous hazard points exist during milking and handling of milk that can lead to fecal contamination, even while using recommended hygienic methods. Contamination with as few as 10 *E. coli* O157 bacteria might be sufficient to cause human infection [24]. Thus, to adequately control the microbial risks of raw milk, we strongly recommend its pasteurization, which substantially decreases or eliminates pathogens and effectively prevents disease transmission [19, 25].

However, in states similar to Connecticut where pasteurization is not required and direct sale of raw milk to consumers is permitted, alternative approaches to pasteurization that would minimize disease transmission are critically needed. In response to this outbreak, the CT DAG proposed legislation supported by the CT DPH that included 3 alternative control measures. The first measure called for strengthening of labels from the current advisory to a detailed warning highlighting groups of persons at highest risk for experiencing disease (eg, children and elderly persons); the label would be prominently displayed on all raw milk containers. Whereas some raw milk enthusiasts might be aware that raw milk can harbor disease-causing bacteria, other consumers might not. As revealed in a postinvestigation discussion with a case patient, even if consumers recognize that inherent hazards might exist with raw milk, they might not appreciate the potential severity of such hazards,

particularly for young children. Thus, a detailed warning label would allow consumers to make a more informed decision.

The second measure proposed by the CT DAG consisted of increasing the frequency of raw milk testing for human pathogens from a quarterly to monthly basis and lowering the coliform threshold from ⩽50 CFU/mL to ⩽10 CFU/mL. We know raw milk testing alone is not sufficient to eliminate all risk for exposure because test results only reflect the conditions at the time of sampling; any contamination that occurs afterward will not be detected. However, raw milk testing can reduce the extent of an outbreak. Although weekly testing for coliform bacteria by farm X did not prevent this outbreak, the test results eventually led to a voluntary suspension of raw milk production, thus minimizing potential exposures of more consumers.

The last measure proposed was limiting raw milk sale to farm premises. Of the 14 case patients, 3 had purchased raw milk at retail markets, and an additional 2 experienced disease from 1 of these 3 patients as a result of secondary and tertiary transmission, respectively. Prohibiting nonfarm retail sale of raw milk might have prevented at least 36% of the cases in this outbreak. In addition, because of similar packaging of raw and pasteurized milk, consumers might inadvertently purchase raw milk in a retail market. Limiting sale to farm premises can protect certain consumers while allowing raw milk enthusiasts to continue purchasing raw milk. Implementing the described labeling requirement would at least ensure that these raw milk enthusiasts would be aware of the potential risks associated with their purchases. Requiring consumers to visit a farm and having the farms maintain a customer list might also facilitate case finding during investigations of future raw milk–associated outbreaks. Lastly, consumers can also develop a relationship with the milk producer that might include a better understanding of how milk is produced and thus realize the inherent difficulties of ensuring raw milk safety through hygienic measures alone.

Almost one-half of all persons who had consumed raw milk in the household case-control study experienced illness. Although not all ill persons developed severe disease, 4 of the 5 hospitalized case patients experienced HUS or TTP. The *E. coli* O157:NM isolates in this outbreak produced Shiga toxin 2, which is reportedly more virulent than Shiga toxin 1 [26]. Certain Shiga toxin 2 subtypes and other putative virulence factors also have been postulated to be responsible for increased pathogenicity [27]. Although we did not test for other factors, it is possible that unidentified strain-specific determinants might have contributed to the increased virulence observed in this outbreak.

The total outbreak cost is likely underestimated. The medical expenses do not include costs of inpatient consultation by private physicians, costs of outpatient visits and over-the-counter medications, and travel costs associated with medical visits. Although >50% of medical expenses reflected the amount charged rather than the actual cost of care for the 1 patient with TTP, the overall medical costs of the other 4 patients were still substantial, and a bulk of the costs was likely a result of dialysis performed for HUS treatment. Patients who experienced HUS or TTP might continue to incur long-term treatment costs. In addition, we did not estimate the lost productivity costs of caretakers. We also did not account for costs that the farm and retail stores might incur from lawsuits and increased insurance premiums.

The public health activities associated with the highest costs were laboratory testing and communication of findings. The nature of this foodborne outbreak required multiple specimens to be tested, generating substantial laboratory and personnel costs. Because of the high proportion of patients who were young children experiencing serious medical complications, this outbreak generated significant media and public attention. Considerable amount of staff time was dedicated to communicating epidemiologic findings and responding to inquiries by concerned parents.

Our study had certain limitations. A selection bias might have existed in our identification of probable cases because the definition used included being a farm X customer. However, because the community case-control study that implicated farm X raw milk as the source was conducted with confirmed cases and excluded probable cases, we believe the association we identified between farm X raw milk consumption and illness was likely valid. In addition, the extent of the outbreak might have been underestimated because the list of farm X customers was incomplete.

As demonstrated by this *E. coli* O157 outbreak, raw milk consumption continues to be hazardous and can be associated with substantial medical and public health costs. In states where pasteurization or a total ban on raw milk sale cannot be enforced because of the strong opposition of raw milk advocates, alternative control measures need to be implemented to protect public health. Although the response at the public hearing with the Environment Committee of the Connecticut State Legislature held on 9 February 2009 was against the passage of the proposed legislation, we remain hopeful that continual attempts through this kind of incremental approach, along with efforts highlighting the potential severity of associated health risks, will ultimately reduce raw milk consumption and the subsequent health hazards.

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