

Knox County Health Department Outbreak Summary
TN18_055: STEC associated with consumption of Raw Milk

Background

Shiga toxin-producing *Escherichia coli* (STEC) can cause an acute diarrheal illness. STEC O157:H7 is the most commonly reported STEC and has been linked to several sources including the consumption of unpasteurized (raw) milk, contaminated produce, improperly cooked ground beef, and contact with ruminant animals. Approximately 5-15% of persons with this infection develop hemolytic uremic syndrome (HUS). Persons at highest risk for HUS are children less than five years of age. The Knox County Health Department (KCHD) investigated a STEC outbreak among persons who consumed raw milk from a local dairy during the spring of 2018.

Methods

Notification:

KCHD launched an investigation after receiving three reports of STEC in children under five years of age. Initial laboratory reports were received by the Tennessee Department of Health (TDH) and KCHD on May 30, 2018 with the case interview being conducted by TDH on May 31, 2018. KCHD was notified by TDH of raw milk exposure on June 1, 2018. KCHD worked closely with the TDH FoodNet and FoodCORE programs to interview cases, ensure clinical isolates and environmental samples were sent to the Tennessee Department of Health-Public Health Laboratory in Nashville (TNPHL-Nashville), conduct active case finding and an environmental investigation, and consider any possible exposures and/or links between cases.

On initial interview, all cases reported drinking raw milk from French Broad Farm (Dairy X).

Public Health Actions:

Dairy X was contacted on June 3, 2018, regarding the outbreak of *E. coli* O157:H7 with suspected linkage to the consumption of raw milk from their facility. A request for a list of all cow share members and their contact information was made in order to conduct active case finding as soon as possible and to warn consumers about the potential of a contaminated product. (Cow share programs allow for members to buy part or all of a cow; members are legally entitled to access raw milk from their cow).

On June 5, 2018, the health officer from KCHD provided a health directive to Dairy X requesting they stop the distribution of all raw dairy products, provide a list of phone numbers and email addresses for all cow share participants, and allow health department staff to collect milk and environmental samples, including manure, for testing.

On June 5, 2018, KCHD distributed a media release requesting anyone with milk from Dairy X discard all unpasteurized milk products that they may have and seek medical attention if they are ill.

A list of 207 cow share participants was obtained on June 5, 2018, and KCHD activated the Public Health Investigation Team (PHIT) to conduct case finding through telephone interviews and to ask cow share participants to discard any raw milk products they may have.

A phone bank, staffed by the PHIT, was set up on June 6, 2018, to field any calls from the public regarding the outbreak.

Environmental Methods:

Visit to Dairy X: On June 5, 2018, an environmental health specialist from KCHD delivered the health directive to the owner of Dairy X. The environmental specialist confirmed that all raw milk production and distribution operations would stop until further notice.

Raw Milk Sample Collection: On June 5, 2018, the environmental health specialist collected one unopened gallon container of raw milk from the dairy.

Four raw milk samples from household 1 (HH1) were collected on June 5, 2018, and one whole gallon of unopened milk was collected from household 2 (HH2) on June 5, 2018. Both households were members of the cow share.

On June 6, 2018, a Tennessee Department of Agriculture inspector, accompanied by the KCHD environmental health inspector, collected two additional raw milk samples from the raw milk bulk tank at Dairy X.

Cow Manure Sample Collection: On June 5, 2018, the environmental health specialist attempted to retrieve cow manure samples from Dairy X but was refused access to the farm by the owner. A member of the dairy owner's family met with the environmental health specialist and scheduled a time on June 6, 2018, to allow access for environmental sampling.

The environmental health specialist collected three fresh composite manure samples from three sites around the farm. Manure samples were collected with a sterile tongue depressor and placed in sterile Whirl-Pak bags.

All environmental and animal samples were transferred to the Tennessee Public Health Laboratory in Knoxville (TNPHL-Knoxville) using the KCHD chain of custody form. Please see **Table 6** for a summary of environmental samples collected.

Epidemiologic Methods:

Case Interviews: Confirmed cases were identified through routine reportable disease surveillance. Probable cases were found through calls to cow share participants (active case finding) or through completed REDCap surveys that were sent to cow share participants via e-mail or text message. All cases were interviewed using the standardized STEC case report form per TDH surveillance procedures.

Case Definitions: During the initial phase of the outbreak, the case definition was broad to capture anyone who was reported as having STEC. As knowledge about common exposures was obtained through case interviews and laboratory evidence became available, the final case definition was narrowed to the following:

- **Confirmed case:** A person with laboratory confirmation of STEC O157:H7 PFGE pattern EXHX01.0047/EXHA26.3187 or shiga toxin positive lab results between May 20, 2018, and June 4, 2018, who resides in Knox County or the East Tennessee Region and reports consuming raw milk from Dairy X.
- **Probable case:** ≥ 2 days of diarrhea between the dates of May 20, 2018, and June 4, 2018, plus receives milk from Dairy X or epi-linked to a confirmed case (Diarrheal illness is defined as having 3 or more loose stools in a 24-hour period).

Case Finding: A REDCap questionnaire was developed by the TDH from a modified version of the standardized STEC case report form. This web-based questionnaire was sent to cow share participants via a Tennessee Health Alert (THAN) text message. The link was sent out twice in this format on June 8 and June 14, 2018. The survey link was then sent to e-mail addresses of individuals who had not yet responded to the survey on June 18, 2018. Information including demographics, illness, and food and animal exposures was requested for each member of the household.

Results from case finding activities were analyzed using Epi-Info 7 and Microsoft Excel. Binomial probability was conducted using the 2007 FoodNet Atlas of Exposures.

Laboratory Methods:

Milk and Environmental Samples: TNPHL-Knoxville shipped raw milk samples (6), milk jugs (2), and cow feces samples (3) to the United States Department of Agriculture (USDA), Agricultural Research Service (ARS). Culture of Shiga-toxigenic *Escherichia coli* O157:H7 was attempted at USDA using the methods described here:

http://www.chromagar.com/fichiers/1283757285EF_Outbreak_investigation_STEC_O157_MLO_2010_03.pdf. Suspected O157:H7 isolates were shipped back to TNPHL-Nashville for confirmation.

Clinical Samples: Stool specimens were collected from 10 ill children from primary care providers and a local children's hospital. Stool was tested at the TNPHL-Nashville for shiga toxin by Polymerase Chain Reaction (PCR) and *E.coli* O157:H7 by culture. Pulsed-Field Gel Electrophoresis (PFGE) analysis was performed on viable clinical samples.

Clinical and environmental samples yielding a STEC isolate were prioritized for PFGE and whole genome sequencing (WGS) at the TNPHL-Nashville, according to PulseNet protocols. WGS analysis was conducted by the Centers for Disease Control and Prevention (CDC) using a high-quality single-nucleotide polymorphism (hqSNP) methodology.

Results

Public Health Measures:

KCHD PHIT attempted to contact 207 cow share participants from the cow share list provided by Dairy X (**Table 1**). Of the 207 individuals, 166 had a phone number; 41 cow share participants did not have any contact information listed. Of the 69 individuals who answered the phone, six stated that someone in

their household had been experiencing symptoms consistent with STEC illness. A standard STEC interview form was completed for individuals who met the probable case definition.

Table 1: Summary of PHIT Calls Made to Cow Share Participants

Telephone Contact Status	Count
No Phone Number Listed	41
Unable to leave a message (no identifier in voicemail)	39
Wrong Number	13
Left a voicemail message	45
Spoken with and provided guidance on discarding milk and education about illness	69
Total Call Attempts	207

The phone bank set up on June 6, 2018, was closed that evening after receiving a total of 15 calls. It was determined that the call volume could be handled through standard public health inquiry procedures.

On June 14, 2018, the health directive was lifted from Dairy X since no new cases of STEC with an epidemiological link were reported.

Environmental Assessment:

As of August 8, 2018, the dairy had not resumed distribution of raw milk. All milk produced at the dairy farm was being processed to achieve pasteurization. KCHD shared safe milking practices, procedures, documents and guidance from the University of Tennessee Agriculture Extension with the dairy. Consultation services with the Agriculture Extension were offered and available upon the farmer’s request.

Epidemiologic Results:

Case Interviews: A total of 17 cases were identified: 10 confirmed cases, and seven probable cases. Confirmed cases ranged in age from 1 to 16 years, with a median age of 2.4 years. Probable cases ranged in age from 1 to 39 years with a median age of 1.8 years. Of the 10 confirmed cases, seven cases were hospitalized and six were diagnosed with hemolytic uremic syndrome (HUS) (See **Table 2**). Illness onset dates for confirmed and probable cases ranged between May 22 and June 4, 2018 (**Figure 1**).

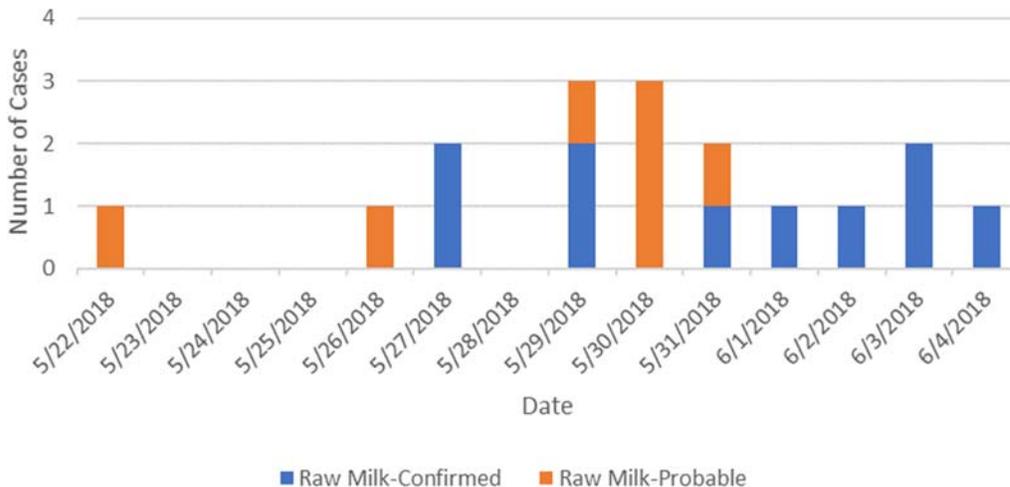
Table 2: Case Characteristics and Summary of Clinical Information

Case Characteristics		Confirmed Cases	Probable Cases
Total		10	7
Age*			
Age Range		1.06 – 16.74 Years	1 - 39
Average Age		4.28	10.87
Median Age		2.40	1.75
Gender			
Males		7	1
Females		3	6
Medical			
Hospitalized		7	0
Average Length of Hospital Stay		16 days (range: 1-50 days)	N/A
Median Length of Hospital Stay		13 days (range: 1-50 days)	N/A
HUS Diagnosis		6	0
Duration of Illness		N/A	3 days**
Symptoms		Confirmed Cases	Probable Cases
Diarrhea		9	7
Bloody Diarrhea		8	1
Vomiting		4	0
Fever		4	2
Abdominal Pain		3	0
Nausea		1	2

* Age was available for 4 of 7 probable cases

** Duration of illness was available for 5 of 7 probable cases

Figure 1: Epidemiologic Curve of Illness Onset Date, May 22, 2018-June 4, 2018



Case Finding: A RedCap Survey link was sent to 67 e-mail addresses and via text message to 143 telephone numbers. The survey completion rate was 16.9%. The 35 households that completed the RedCap survey included 49 individuals (some households completed a survey for more than one household member). The survey identified seven ill persons who met the probable case definition and 41 well persons.

Of the 10 confirmed cases, four reported picking up raw milk on May 25, 2018, two picked up on June 1, 2018, and four picked up on both dates. Consumption of milk following both sets of pickup dates is consistent with the incubation period and onset of illness (**Figure 1**).

Table 3: Summary of RedCap Surveys Sent

Survey Status*	
Text Messages containing survey link sent	143
Reminder text messages containing survey link sent	128
E-mails containing survey link sent	67
Completed Surveys	49

**Some individuals received both text message and e-mail*

Binomial Probability: The Foodnet Atlas of Exposure quantifies food consumption habits among healthy individuals in FoodNet sites across the US, including Tennessee. In the 2006-07 version of this survey, 3.5% of healthy Tennessee residents reported consuming raw milk in the seven days prior to the survey. The consumption rate among confirmed cases in this outbreak was 100%. Comparison of these numbers using a binomial probability calculation yielded a p-value of <0.00001, suggesting the consumption rate of raw milk among outbreak cases was much higher than the consumption rate in the overall population. Although a similar calculation was conducted for individuals consuming yogurt, sources of yogurt listed by cases in this outbreak were from five different companies. Additional exposures were evaluated using this same method and the results are summarized in **Table 4**.

Table 4: Summary of Exposures Commonly Associated with *E. coli* Among Cases.

Exposures reported by confirmed cases (N=10)	Number of Cases who Answered Question	Yes (%)	Maybe (%)	Yes or Maybe (%)	TN Foodnet Survey Consumption Rate	Binomial Probability P-value
Live, work, visit a farm/petting zoo	10	1 (10%)	0	1 (10%)	5.07%	0.3
Cats	9	1 (33%)	0	1 (33%)	39%	0.07
Dogs	10	6 (60%)	0	6 (60%)	63.8%	0.243
Chicken/turkey	10	1 (10%)	0	1 (10%)	3.6%/1.3%	0.26
Rodents/small mammals	9	1 (11%)	0	1 (11%)	Data not available	Data not available
Any beef	9	5 (56%)	0	5 (56%)	Data not available	Data not available

Ground beef (inside or outside home)	8	4 (50%)	1 (13%)	5 (63%)	44.9%	0.26
Steak or roast beef	8	2 (25%)	0	2 (25%)	47.5%	Data not available
Handled beef in the home (you or someone else)	8	4 (50%)	0	4 (50%)	Data not available	Data not available
Pork	8	4 (50%)	0	4 (50%)	47.2%	0.27
Bison	7	1 (14%)	0	1 (14%)	Data not available	Data not available
Wild game	7	1 (14%)	0	1 (14%)	5.6%	0.28
Dried meats	7	3 (43%)	0	3 (43%)	Data not available	Data not available
Other meat	8	2 (25%)	2 (25%)	4 (50%)	Data not available	Data not available
Pasteurized milk	10	4 (40%)	0	4 (40%)	78.2%	0.008
Raw or unpasteurized milk	10	10 (100%)	0	10 (100%)	3.5%	0.000000
Yogurt	9	9 (100%)	0	9 (100%)	38.9%	0.0002
Ice cream	9	7 (78%)	0	7 (78%)	62.2%	0.19
Cheese (any)	9	6 (67%)	0	6 (67%)	53.3%	0.2

Source: The Foodnet Atlas of Exposure (<https://www.cdc.gov/foodnet/surveys/population.html>)

Laboratory Results:

Clinical Samples: Clinical specimens were collected from 10 cases. All 10 specimens tested positive for shiga toxin 2. STEC O157:H7 was isolated from nine cases. One isolate was not viable for culture. The nine isolates were characterized as STEC O157:H7 with the same PFGE pattern (XbaI pattern EXHX01.0047, BlnI pattern EXHA26.3187). Detailed results are presented in **Table 5**.

Environmental and Milk Samples: Two cow feces specimens collected from the farm were shiga toxin positive and yielded E.coli O157:H7 by culture. These isolates were indistinguishable to the clinical samples by PFGE (XbaI pattern EXHX01.0047, BlnI pattern EXHA26.3187). All milk samples were negative for E.coli O157:H7. Detailed results are presented in **Table 6**.

Household Milk Samples: Milk samples obtained from HH1 and HH2 (Dairy X) were negative for Shiga toxin and *E. coli* O157 was not isolated. (**Table 6**).

Dairy Milk Samples: Milk samples obtained from the farm tested by TNPHL-Nashville, and USDA were all negative for STEC (**Table 6**).

Dairy Manure Samples: Initial testing of two of the three manure samples yielded presumptive positive results at USDA. After receipt of the two suspected isolates from the USDA to TNPHL-Nashville, *E. coli*

O157:H7 was confirmed and PFGE pattern was indistinguishable from the outbreak pattern (Xbal pattern EXHX01.0047, BlnI pattern EXHA26.3187) (Table 6).

All clinical and environmental isolates were highly related by WGS (within 0-3 SNPs).

Table 5: Laboratory Results for Clinical Samples

NBS PID	Culture Date	Accession	PFGE Xbal	PFEG BlnI	TNPHL-Nashville Culture Result
3683521	5/30/18	N18E163805-01	EXHX01.0047	EXHA26.3187	STEC O157:H7 STX 2
3687511	5/31/18	N18E166886-01	Not Viable	Not Viable	Not Viable
3687239	6/2/18	N18E166539-01	EXHX01.0047	EXHA26.3187	STEC O157:H7 STX 2
3687287	6/2/18	N18E166536-01	EXHX01.0047	EXHA26.3187	STEC O157:H7 STX 2
3687335	6/3/18	N18E166537-01	EXHX01.0047	EXHA26.3187	STEC O157:H7 STX 2
3692459	6/3/18	N18E171842-01	EXHX01.0047	EXHA26.3187	STEC O157:H7 STX 2
3692456	6/3/18	N18E171841-01	EXHX01.0047	EXHA26.3187	STEC O157:H7 STX 2
3689605	6/4/18	N18E171650-01	EXHX01.0047	EXHA26.3187	STEC O157:H7 STX 2
3695198	6/4/18	N18E168408-01	EXHX01.0047	EXHA26.3187	STEC O157:H7 STX 2
3705669	6/13/18	N18e187044	EXHX01.0047	EXHA26.3187	STEC O157:H7 STX 2

Table 6: Laboratory Results for Environmental Samples

TN Codes	Sample Source	Sample Type	USDA-Nebraska Culture Result	Accession	PFGE Xbal	PFEG BlnI
TN18-055-01	HH2	Milk aliquot from full unopened gallon	Negative			
TN18-055-02	HH2	Milk from opened gallon	Negative			
TN18-055-03	HH1	Milk jug	Negative			
TN18-055-04	HH1	Milk jug	Negative			
TN18-055-05	HH1	Milk aliquot from full unopened gallon	Negative			
TN18-055-06	HH1	Milk aliquot from full unopened gallon	Negative			
TN18-055-07	Dairy	Milk sample	Negative			
TN18-055-08	Dairy	Milk sample	Negative			
TN18-055-09	Dairy	Cow feces from raw milk dairy	Presumptive Positive	N18E178069-01	EXHX01.0047	EXHA26.3187
TN18-055-10	Dairy	Cow feces from raw milk dairy	Presumptive Positive	N18E178074-01	EXHX01.0047	EXHA26.3187
TN18-055-11	Dairy	Cow feces from raw milk dairy	Negative			

Discussion

Ten confirmed cases and seven probable cases were identified during the course of this investigation. All 10 confirmed cases reported consumption of raw milk in the seven days prior to illness onset. This rate of raw milk consumption is much higher than would be expected among the general population (3.5%). Statistical methods suggest this difference is so strong (100% vs. 3.5% expected population consumption) that raw milk is the likely source of illness.

Case finding activities did not yield optimal controls for conducting a cohort study since everyone in the cohort had exposure to raw milk. The purpose of the study was to identify additional exposures and other risk factors (age, amount of milk consumed, batch of milk consumed) that could have contributed to some raw milk drinkers becoming ill while others did not. Unfortunately, due to a very low response rate, the case control study was not able to adequately evaluate these additional risk factors.

Duration of illness could not be calculated for cases since many cases had long-term effects from their illness.

The conclusion that raw milk was the source of illness during this outbreak is not only supported by the epidemiology but by the identification of the same outbreak strain in patients and in cow feces collected from Dairy X. The patient isolates and farm isolates were virtually identical by whole genome sequencing.

Consuming raw milk comes with a probable risk of consuming pathogens such as E.coli that would otherwise be eliminated through pasteurization. Raw milk cow shares are legal in the state of Tennessee and offer a challenging environment for local jurisdictions to investigate. At this time, it is unknown whether the dairy will decide to redistribute raw milk through cow shares in the future.

Conclusions

An *E. coli* O157:H7 outbreak associated with consumption of raw milk from Dairy X was identified. Multiple lines of evidence implicated the product including analytical epidemiology, an epidemiologic traceback, and laboratory evidence isolating the outbreak strain of STEC O157 from cow manure collected from the dairy and from clinical specimens.

Recommendations

- To reduce the risk of foodborne illness, all milk should be pasteurized.
- Children, especially those five years old and younger, should not be given raw milk due to the risk of infection and complications including HUS and death.
- Those who participate in cow share programs should be educated about the risks associated with raw milk and who is at greatest risk.

Limitations

- All individuals responding to the survey had consumed raw milk thus limiting the exposure criteria required to conduct an adequate odds ratio.
- Binomial probability serves as one indicator of probability that raw milk contributed to the outbreak, however, the Foodnet Atlas of Exposure was published in 2007 and consumption rates of raw milk in Tennessee may have changed.

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