

**Outbreak of Shiga toxin-producing Escherichia coli (STEC) O157:H7  
Associated with a Kansas Cider Festival — Eastern Kansas, 2016**



## **Background:**

On Friday, October 21, 2016 at 4:56 P.M., the Kansas Department of Health and Environment (KDHE) was notified by the Missouri Department of Health and Senior Services (MDHSS) of 4 Kansas residents and 2 Missouri residents with reported cases of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 which were indistinguishable by pulsed-field gel electrophoresis (PFGE). Persons diagnosed with STEC, including STEC O157:H7, are interviewed using a standard questionnaire to identify potential sources of infection. A review of these questionnaires found 5 of the 6 persons attended the Louisburg Cider Mill Cider Festival (“festival”) during their incubation period. At 11:33 A.M. on Monday, October 25, 2016, KDHE notified MDHSS, Kansas Department of Agriculture (KDA), Miami County Department of Health, Johnson County Department of Health and Environment, and the Kansas City District Office of the Food and Drug Administration (FDA) of the potential connection to the festival. An outbreak investigation was initiated to determine the cause of the illness, scope of illness, and to implement prevention and control measures.

## **Methods:**

### Epidemiologic Investigation

Standard questionnaires and PFGE for persons with STEC infection reported after September 1, 2016 were reviewed to identify additional persons who had exposure to the festival or indistinguishable PFGE. Persons with STEC infection who reported attending the festival were interviewed with an open-ended questionnaire about food and activities at the festival. An in-depth outbreak-specific questionnaire, focused on food and activities at the festival, was developed based on interviews and an environmental assessment.

A matched case-control study was conducted to determine potential associations between illness and festival exposures. A press release sent out by KDHE requested persons who experienced diarrheal illness after attending the festival on September 24 — 25, 2016 or October 1 — 2, 2016 self-report their illness by calling the KDHE epidemiology hotline. Persons reporting illness and friends or family who attended the festival with the ill person were interviewed with the outbreak-specific questionnaire.

For this investigation, a confirmed case was defined as laboratory evidence of STEC serotype O157:H7 with a PFGE pattern or genetic profile indistinguishable from the other confirmed cases. A probable case was defined as laboratory evidence of STEC without PFGE or self-reported diarrhea (three or more loose stools in 24-hour period) in a person who attended the festival beginning at least one day after attending the festival on either September 24 — 25, 2016 or October 1 — 2, 2016 and lasting at least two days. Persons self-reporting gastrointestinal symptoms not meeting case definition were excluded from the analysis. Control subjects were friends and family who reported no gastrointestinal symptoms and attended the festival with a case-patient. Case-patients and control subjects who attended the festival together were matched by family-and-friend group for analysis.

Analysis was conducted using SAS® 9.3. Descriptive statistics including case-patient demographics, symptoms, incubation, and duration of illness were calculated. Matched odds ratios (OR) with 95%

confidence intervals (95% CI) were calculated using conditional logistic regression with exact estimation to determine the associations between exposures at the festival and subsequent illness.

### Laboratory Analysis

Stool specimens were cultured, serotyped, and PFGE was performed on the bacterial isolates at the KDHE or MDHSS Laboratories. Multiple Locus Variable-number Tandem Repeat Analysis (MLVA) was conducted by the Centers for Disease Control and Prevention on a subset of nationwide PFGE matches to better classify the outbreak strain.

### Environmental Assessment

KDA was joined by KDHE epidemiologists and FDA investigators to conduct an environmental assessment of the Louisburg Cider Mill on October 27, 2016. Environmental samples including swabs of potentially contaminated surfaces, cider, and apples were collected and tested for *E. coli* O157:H7 and other STEC serogroups by the KDA laboratories. Additional food samples of cider, apples, and apple butter were submitted by case-patients and tested for *E. coli* O157:H7 at KDA laboratories. Food traceback was completed for implicated food items.

### **Results:**

#### Epidemiologic Investigation

The initial cluster of case-patients with indistinguishable PFGE patterns included four residents of Johnson County, Kansas and two residents of Cass County, Missouri. The two Missouri residents and three of the four Kansas residents reported visiting Louisburg Cider Mill September 24, 2016. Review of the Kansas electronic disease surveillance system identified an additional three Kansas residents diagnosed with STEC who attended the festival on September 24 — 25, 2016. These additional three ill persons included: one person diagnosed using culture-independent diagnostic testing (CIDT) at private lab but not confirmed at KDHE laboratories; one person diagnosed with STEC following diagnosis of hemolytic uremic syndrome (HUS; a life-threatening complication of STEC infection); and one person who had a unique PFGE pattern found to be similar to the cluster PFGE. No additional cases of the unique PFGE pattern were identified. MDHSS notified KDHE of an additional ill person with an indistinguishable PFGE pattern who did not report attending Louisburg Cider Mill. Interview of persons who self-reported illness to the KDHE epidemiology hotline identified an additional Missouri resident with CIDT-diagnosed STEC.

A total of nine persons diagnosed with STEC and reported to KDHE were found to have attended the festival. Five of the nine had indistinguishable PFGE patterns and one had closely-related PFGE pattern.

Two persons with indistinguishable PFGE patterns did not report visiting the Louisburg Cider Mill. Both ill persons reported they ate apples bought from local grocery markets (Grocery A and Grocery B) prior to illness.

KDHE and MDHSS conducted 133 interviews with persons who reported visiting the Louisburg Cider Mill. Sixteen persons, 11 of whom were ill, reported attending the Louisburg Cider Mill on a date other than the festival weekends and were excluded from analysis. An additional six ill persons were excluded from the analysis: two reported no diarrhea, two started having diarrhea the same day they attended the festival, and two had diarrhea lasting less than 2 days.

The remaining 111 persons were matched according to the friends-and-family group with whom they attended the festival. There were 41 friend-and-family groups, among which were 56 ill persons and 55 non-ill persons. The predominant symptoms other than diarrhea were abdominal pain and nausea (Table 1).

Among ill persons, 17 (30%) reported visiting a healthcare provider, 11 (20%) reported visiting an emergency room, 10 (18%) reported being hospitalized for their illness, and 2 (4%) developed HUS (Table 1). The majority of interviewed ill persons were female (n=38), Kansas residents (n=37), and attended the festival on September 24, 2016 (n=32); these characteristics were similar to those of non-ill interviewed persons.

**Figure 1: Number of cases by onset date of illness – Outbreak of *E. coli* O157:H7 associated with Louisburg Cider Mill festival, September – October 2016 (n=56)**

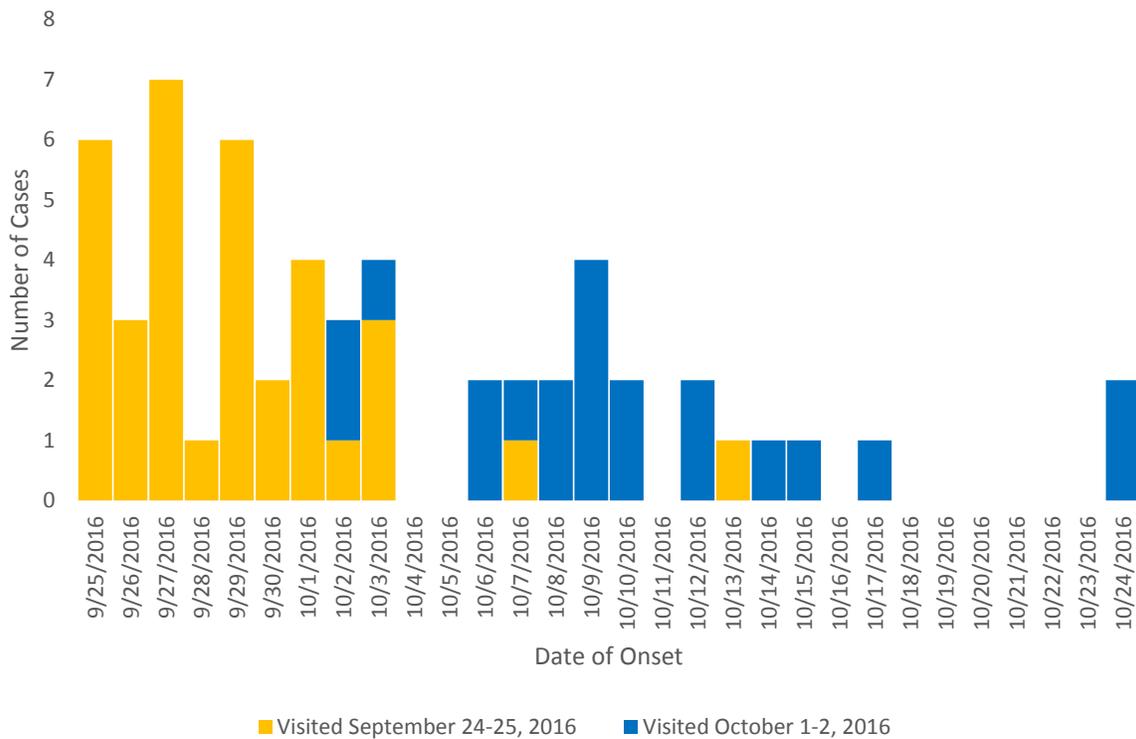


Table 1. Symptoms reported among ill persons (n=56)

Symptom	Number of ill persons	Percent of ill persons
Diarrhea	56	100%
Blood in stool	14	25%
Abdominal pain	43	77%
Nausea	32	57%
Vomiting	16	29%
Fever	23	41%
Myalgia	18	32%
Headache	20	36%
Other symptoms	16	29%
Visited healthcare provider	17	30%
Emergency room	11	20%
Hospitalized	10	18%
Hemolytic uremic syndrome	2	4%

Table 2. Characteristics of ill and non-ill interviewed festival attendees

	Ill persons, n=56 n (%)	Non-ill persons, n=55 n (%)
Median age	22	29
Sex		
Male	18 (32%)	26 (47%)
Female	38 (68%)	29 (53%)
State of residence		
Kansas	37 (66%)	37 (67%)
Missouri	19 (34%)	18 (34%)
Date of attendance		
September 24, 2016	32 (57%)	36 (65%)
September 25, 2016	3 (5%)	2 (4%)
October 1, 2016	5 (9%)	4 (7%)
October 2, 2016	16 (29%)	12 (24%)

Drinking cold cider (exact OR: 10.3; 95%CI: 1.2-infinity), either as slush, a frozen cider drink, or iced cider, and eating doughnuts (OR: 10.3; 95%CI: 1.1-94.8) were significantly associated with illness. None of the activities or vendors was significantly associated with illness (Table 3). The majority of friend-and-family groups reported similar cider and cider doughnut exposure among all members of the group. However, among the 7 friend-and-family groups that did not all report drinking cider, 100% of the persons who drank cider were ill and 100% of the persons who did not drink cider were not ill. Among a different set of 7 friend-and-family groups who did not all report eating cider doughnuts only 85% of ill persons reported eating cider doughnuts.

**Table 3. *E. coli* O157:H7 exposure information**

Exposure/Food item	Number of control subjects n = 55 n(%)	Number of case-patients n = 56 n(%)	OR	95% CI
<b>Any type of cold cider</b>	<b>36 (65)</b>	<b>41 (73)</b>	<b>6.6</b>	<b>1.2 - infinity</b>
cup of cold cider	19 (35)	17 (30)	2.1	0.4 - 11.6
cider slush	21 (38)	26 (46)	4.5	0.7 - infinity
Hot cider	4 (7)	7 (13)	1.2	0.2 - 8.6
Pre-packaged bottle of cider	15 (27)	14 (25)	3.3	0.3 - 39.1
<b>Doughnuts</b>	<b>48 (87)</b>	<b>52 (93)</b>	<b>10.3</b>	<b>1.1 - 94.8</b>
Caramel apples	3 (5)	5 (9)	1.3	0.1 - 17.3
Pony ride	1 (2)	0 (0)	1.0	0 - 19
Contact with farm animals	7 (13)	7 (13)	4.7	0.5 - infinity
Pumpkin patch	21 (38)	25 (45)	2.2	0.1 - 157
Eating at mobile food vendor	32 (58)	31 (55)	0.3	0.3 - 2.4

#### Laboratory Analysis

Ten of the case-patients who attended the festival had stool specimens tested for STEC. Seven of these individuals had the *Xba*I/*Bln*I pattern combination EXHX01.0047/EXHA26.3187, 1 person had *Xba*I/*Bln*I pattern combination EXHX01.6535/EXHA26.4766. The other two persons were positive for Shiga toxin by CIDT but were not confirmed by culture (therefore PFGE was not possible). Two additional persons with a matching EXHX01.0047/EXHA26.3187 PFGE pattern were identified who did not attend the festival; these persons reported exposure to apples from two local grocery markets (grocery chains A and B).

Four of these isolates from case-patients (including the unique *Xba*I/*Bln*I pattern and a person who did not report visiting Louisburg Cider Mill) were genetically indistinguishable by MLVA; MLVA was not performed on the remaining isolates. No additional isolates with indistinguishable PFGE submitted for MLVA testing from other states were genetically linked to the outbreak.

#### Environmental Assessment

There were 10 environmental samples collected during the October 27, 2016 environmental assessment of the Louisburg Cider Mill; all samples were negative for STEC, including *E. coli* O157:H7. Apples and prepackaged cider taken from the Louisburg Cider Mill on October 27, 2016 were negative for *E. coli* O157:H7. Additionally, apples purchased on September 25, 2016 submitted by a case-patient were negative for *E. coli* O157:H7. Apple butter and cider purchased on October 1, 2016 submitted by a case-patient were negative for *E. coli* O157:H7.

An inspection of the Louisburg Cider Mill by KDA, KDHE, and FDA was performed. The Louisburg Cider Mill's processes for apple disinfection, milling, pasteurization, bottling, and food service were observed. The Louisburg Cider Mill operates an on-site county store that serves hot cider, cold cider, cider slushes, and cider doughnuts year-round. Outdoor food stands also serve cider and food during the festival. No food code violations were noted for the production process or food service. There were no issues

identified with either the cold-press flash pasteurization process (cider is brought above 160°F/70°C for 20 seconds) or general pasteurization (cider is brought above 160°F/70°C for 20 – 30 minutes). All cider products were pasteurized before bottling. Louisburg Cider Mill's policy is to use bottled, pasteurized cider at the country store and food stands and as an ingredient in cider doughnuts. During periods of high customer demand, such as during the festival, employees sometimes filled five-gallon buckets from a chilled cider tank and used that cider rather than the bottled product. The production manager identified that the chilled cider tank used to fill the 5-gallon buckets was unpasteurized. The chilled cider tank is inside the building where bottling and food preparation occurs and stores chilled cider prior to flash pasteurization. This is the only tank inside the building identified as holding un-pasteurized cider and is located near where cider doughnuts are made and the county store. Other cider storage tanks reported by Louisburg Cider Mill to hold un-pasteurized cider are located outside the building. Additional cider storage tanks reported by Louisburg Cider Mill to hold pasteurized cider were located at the far end of the building away from food preparation. The practice of using cider from the chilled tank was not observed during the environmental assessment. Louisburg Cider Mill staff could not definitively state what dates and times unpasteurized cider was served from the chilled tank, or for how many years that process had been in place, only that it was not a routine process but did occur during high-volume periods.

The apples at the Louisburg Cider Mill were delivered from 5 orchards (2 in Missouri, 2 in Illinois, and 1 in Washington) in the days prior to and during the festival. Contracts with apple orchards delivering apples to the Louisburg Cider Mill prohibit delivery of dropped apples (i.e. apples picked up off the ground at the orchard). Apples are trucked to the Louisburg Cider Mill by a single trucking company using a dump truck. The traceback of apples delivered through the trucking company identified that the trucks carried pig iron, rock, sand, and apples prior to picking up and delivering apple shipments to Louisburg Cider Mill. An inspection of the orchard delivering the majority of apples during the festival was conducted by the Missouri Department of Agriculture (MDA) in September 2016. This inspection found no violations. Additionally, the MDA inspector noted no dropped apples were known to have been shipped to the Louisburg Cider Mill from this orchard.

The two persons who reported no exposure to the Louisburg Cider Mill reported shopping at two grocery chains (Grocery A and Grocery B) and purchasing apples. KDHE received shopper card information for one of the two persons who reported shopping at Grocery A and was unable to confirm an apple purchase. Grocery B does not have a shopper loyalty program. Apple shipment records for Grocery A recorded deliveries during September – October, 2016 from 2 of the apple orchards also supplying apples to Louisburg Cider Mill. Apple shipment records for Grocery B recorded deliveries during September – October, 2016 from an apple orchard supplying apples to both Grocery A and Louisburg Cider Mill. No additional apple orchards were reported to have shipped to Louisburg Cider Mill and Grocery A or Grocery B.

## Conclusion:

This was an outbreak of STEC O157:H7 associated with the Louisburg Cider Mill during their annual cider festival September 24 — 25, 2016 and October 1 — 2, 2016. Forty-eight persons self-reported gastrointestinal illness after attending the festival in addition to the 8 persons initially identified through review of KDHE and MDHSS communicable disease case surveillance.

There were multiple potential sources of STEC contamination at the festival that have been previously linked to infection including: farm animals, mobile food vendors, and unpasteurized apple cider (1-3). The case-control study analysis identified consumption of cold cider or cider doughnuts as the most likely source of infection, no other potential sources of contamination were statistically associated with illness. Unpasteurized apple juice products, such as apple cider, are a known risk factor for STEC infection. While farm animals are the main reservoir for *E. coli* (4-5), cider mills often use dropped apples, apples found on the ground at the apple orchard, which can be contaminated with manure (2-3). While illness can develop following exposure to as few as 10 bacteria (4), the PH balance, temperature, and sugar content of cider promote *E. coli* growth increasing the likelihood of infection (6). Louisburg Cider Mill had implemented control measures to prevent illness including contracts with apple orchards that specifically excluded dropped apples, and pasteurization of all products intended for sale or consumption. The environmental assessment conducted by KDA found no violations associated with cider production. However, Louisburg Cider Mill reported employees responsible for food service at the onsite country store and food stand sometimes filled 5-gallon buckets from an unpasteurized chilled cider storage tank to keep up with demand for cider products on busy days. While temperature records for mulled cider were not inspected, simmering cider just below boiling at a minimum temperature of 160°F/70°C effectively pasteurizes the product (4-5). Cold cider and cider slushes were served unpasteurized. Louisburg Cider Mill reported more cold cider, either as chilled cider or cider slush, was served on September 24, 2016.

Environmental samples collected on October 27, 2016 from the implicated storage tank, slush machines, and coolers used to serve cider were negative for *E. coli* O157:H7. Thus, this investigation was unable to rule out another source of *E. coli* contamination. Two ill persons whose illnesses were linked by PFGE and MLVA to the outbreak did not report visiting the Louisburg Cider Mill but did report eating apples prior to their illness. A single orchard was identified as having delivered apples to both the Louisburg Cider Mill the day before the festival as well as Grocery A and Grocery B supporting the association between the apples, cider and illness. The majority of illnesses occurred in persons who reported visiting the festival directly after delivery from this orchard including all laboratory confirmed cases of illness. Additionally, all ill persons who visited Louisburg Cider Mill had either cider or a cider doughnut.

The investigation was limited by several factors. The case-control study used a convenience sample of persons who self-reported illness to KDHE and is likely an under-estimate of the true burden of illness as individuals who were less severely ill may not have reported illness. The additional cases identified through persons who self-reported illness to KDHE improved the statistical accuracy of the analysis. Persons who visited the Louisburg Cider Mill on dates outside of the festival who self-reported illness to KDHE were excluded, thus the duration of contamination is unknown. No additional cases of STEC with

indistinguishable PFGE were reported to KDHE other than in the initial cluster of cases. The process that allowed unpasteurized cider to be served to patrons was inconsistent leading to a varying level of unpasteurized cider being served throughout the festival. There was no documentation of when unpasteurized cider may have been served. Additionally, the cider doughnut-making process was not fully observed; while cider is an ingredient in the doughnuts, the use of unpasteurized cider in cider doughnut preparation was not reported by Louisburg Cider Mill. As cooking temperatures are not documented by Louisburg Cider Mill during doughnut preparation, it is unknown whether the doughnuts reached 160°F/70°C, which is the temperature necessary to kill STEC. Fifteen ill persons reported eating cider doughnuts and not drinking cider while only 4 ill persons reported drinking cider and not eating cider doughnuts. This may be due to recall bias as persons were interviewed over a month after attending the festival, however, we cannot rule out potential contamination of the cider doughnuts by an ill employee or by another method of contamination. The investigation did not identify employee illness but employees were not individually interviewed with respect to illness.

This epidemiologic investigation was aided by quick response and cooperation between all partner agencies which allowed for timely initiation of the outbreak investigation once the cluster was identified. The Centers for Disease Control and Prevention performed MLVA on a subset of nationwide specimens to further define illness associated with the outbreak despite a common PFGE pattern. Finally, the Louisburg Cider Mill cooperated with all requests by KDA and KDHE during and after the environmental assessment.

#### **Discussion:**

*E. coli* O157:H7 accounts for approximately 36% of the 265,000 STEC infections reported across the United States annually and is responsible for most of the reported outbreaks of STEC (4). Infection with STEC generally occurs 1 to 10 days after exposure. Symptoms generally start gradually with abdominal pain progressing to severe and often bloody diarrhea lasting generally about a week. Vomiting may also be present but fever occurs less often (5). Hemolytic uremic syndrome (HUS), a severe complication associated with STEC infection, occurs in approximately 5% – 10% of persons infected with STEC. HUS is characterized by acute onset of anemia (destruction of red blood cells) and renal injury or failure. Symptoms generally occur about a week following onset of diarrhea, usually as the patient is beginning to recover, and include low or no urine output, fatigue, unexplained bruising, and decreased consciousness. HUS is fatal in about 5% of persons who develop this complication (7).

STEC is spread through fecal-oral transmission (4) and people can take steps to prevent infection:

- Wash your hands thoroughly
  - After using the bathroom or changing diaper
  - Before preparing or eating food
  - After contact with animals or their environments (at farms, petting zoos, fairs, your own backyard)
  - Anytime they are soiled.

- Cook meats thoroughly. Ground beef and meat that has been needle-tenderized should be cook to a temperature of at least 160°F/70°C. It is best to use a thermometer, as color is not a very reliable indicator of “doneness.”
- Prevent cross contamination of food preparation areas by thoroughly washing hands, counters, cutting boards, and utensils after they touch raw meat.
- Avoid unpasteurized products including dairy products such as raw milk and fresh juices such as apple cider (4).

Citations:

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