

Shiga toxin-producing *E. coli* O111:NM and *Cryptosporidium parvum* Infections Associated with Unpasteurized Apple Cider at an Apple Orchard

October

Washington County

On October 28, 2011, the Minnesota Department of Health (MDH) Public Health Laboratory (PHL) identified two cases of non-O157 Shiga toxin-producing *E. coli* (STEC) with indistinguishable pulsed-field gel electrophoresis (PFGE) patterns (MDH two-enzyme pattern designation ECM425SB400). Illness onset dates were October 11 and October 14.

On initial interview, one case had reported visiting an apple orchard where animals were present, and the other case had reported consuming apples and unpasteurized apple cider at an apple orchard. It was not apparent from the initial interviews if the cases had visited the same apple orchard. An investigation was initiated on October 28.

MDH staff conduct real-time standard interviews with all *Cryptosporidium* and STEC cases identified through routine surveillance. Cases are asked about illness history and exposures prior to illness onset. Completed interviews are reviewed by MDH epidemiologists. Callers to MDH's foodborne illness complaint hotline are also interviewed regarding their illness and exposure histories.

Cases were defined as individuals who either had a stool sample positive for *E. coli* O111:NM with the outbreak PFGE pattern or *Cryptosporidium*, or who became ill with diarrhea (≥ 3 loose stools in a 24-hour period) of at least 3 days duration, after a visit to the apple orchard in October. A secondary case was defined as an individual who met these criteria, lived in the same household as a primary case, and became ill more than 14 days after visiting the orchard.

Cases and their companions who visited the orchard were interviewed with a supplemental questionnaire targeting food, animal, and environmental exposures at the orchard. Contact information for other orchard patrons was not available.

Stool samples were collected from consenting ill patrons and were tested for bacterial and parasitic pathogens at the MDH PHL.

Environmental health (EH) specialists from Washington County Public Health and Environment visited the orchard on October 31 to conduct a kitchen inspection and evaluate potential exposures at the orchard. On November 4, MDH and Washington County staff visited the orchard to obtain stool samples from animals at the on-site petting zoo and environmental samples from the petting zoo area and from inside the apple processing barn. A copy of the *Compendium of Measures to Prevent Disease Associated with Animals in Public Settings, 2009* was provided to the orchard owners.

Animal and environmental samples were tested for STEC, *Salmonella* and *Campylobacter*. Animal samples were also tested for *Yersinia* and *Cryptosporidium*.

Case characteristics

Four cases of *E. coli* O111:NM (*stx1* only) infection with the outbreak subtype were identified through routine surveillance, with dates of illness onset from October 11 to October 15. All had visited the apple

orchard on October 8 (n=2, 50%) or October 9 (n=2, 50%). The median incubation period was 4 days (range, 3 to 6 days). Three (75%) cases were female. The median age of cases was 16 years (range, 6 to 47 years). All four cases reported diarrhea and stomach cramps, two (50%) had blood in their stool, two (50%) had vomiting, and one (25%) had fever. The two cases who had recovered at the time of interview had symptom durations of 4 days and 36 days. None of the cases were hospitalized and none developed HUS.

Three *Cryptosporidium* cases were identified through routine surveillance, with illness onset dates of October 15 (n=2) or October 16 (n=1). Two of the cases had also placed a call to the foodborne illness hotline on October 26 and mentioned visiting the apple orchard on October 9 during their interview. The third case was an individual who matched the outbreak *C. parvum* subtype but who had not mentioned the orchard on initial interview. Upon subsequent interview, the case reported visiting the apple orchard on October 8 or October 9. Two of the *Cryptosporidium* cases were positive for *C. parvum* with subtype BGP16 (IIaA17G2R2); the third case's specimen was not received at the MDH PHL and thus could not be further characterized. The incubation period for the three cases was 6 or 7 days. Two (66%) of the cases were female. The median age of cases was 39 years (range, 31 to 39 years). All three cases reported diarrhea and stomach cramps. One (33%) case had bloody stools and one (33%) had fever. The median duration of illness was 11 days (range, 10 to 12 days). None of the cases were hospitalized.

An ill family member of a laboratory-confirmed STEC case submitted a stool sample to the MDH PHL that tested positive for the outbreak strain of *E. coli* O111:NM and also for *C. parvum* with subtype BGP16a (IIaA17G2R2), one base pair different from the main outbreak strain. The case had visited the orchard on October 9 and had onset of illness on October 17. The case reported diarrhea, vomiting, cramps, and fever. The case's duration of illness was 13 days.

A total of 26 orchard patrons representing 6 independent groups were interviewed. In addition to the eight laboratory-confirmed cases, six additional patrons were identified who met the case definition. Of these six cases, four (67%) were female. The median incubation period was 6 days (range, 2 to 10 days). All of the cases had diarrhea, none had bloody stools, three (50%) had vomiting, and four of four (100%) had stomach cramps. The median duration of illness was 10 days (range, 7 to 12 days).

A secondary case was identified in an ill family member of an STEC case who had onset of illness 16 days after visiting the orchard. Another family member of an STEC case developed symptoms within an hour of visiting the orchard, and another patron reported mild symptoms that did not meet the case definition. All three were excluded from further analyses.

Exposure analysis

All 14 cases were combined for data analysis. By univariate analysis, drinking a sample of unpasteurized apple cider during a pressing demonstration was significantly associated with illness (14 of 14 cases vs. 5 of 9 controls; odds ratio [OR], undefined; $p = 0.01$), and having contact with the petting zoo animals was inversely associated with illness (8 of 14 cases vs. 9 of 9 controls; OR, 0; 95% confidence interval, 0 to 0.8; $p = 0.048$). Neither variable was significantly associated with illness in a multivariate model including both apple cider exposure and animal contact, but small sample size hindered the analysis.

Animal evaluation

The petting zoo at the apple orchard comprised 18 animals: 10 goats, 4 llamas (including 1 cria), 2 sheep, a donkey, and a calf. An MDH veterinarian and other staff from MDH and Washington County

collected fecal samples (digital rectal sample or freshly fallen droppings) from 14 of the 18 petting zoo animals. Samples were not obtained from a goat, a sheep, and two of the llamas. The calf was housed in a separate pen that shared three common fences with the main enclosure. During specimen collection on November 4, the calf had nasal discharge and visible evidence of recent scours. All other animals intermingled in the main pen. Orchard visitors were not allowed to enter the animal enclosure but were able to feed and contact all animals through fences.

STEC was found in 10 of 14 (71%) animal samples. Four animal samples were positive for *stx1* by PCR; isolates from three of the goats were identified as *E. coli* Ound:Hund (n=2) and Ound:H6 (n=1); an isolate was not found from the fourth goat sample. *E. coli* O111:NM subtype ECM425SB415 isolated from the calf sample matched the outbreak pattern found in all five of the human *E. coli* O111:NM isolates by digest with *XbaI* and was one band different by *BlnI*.

Three animal samples were positive for *stx2* by PCR; *E. coli* Ound:H45 was isolated from two goat samples and Ound:H11 was isolated from a llama sample.

A goat and a llama both tested positive for *stx1* and *stx2*. *E. coli* Orough:NM was isolated from the goat sample; an isolate was not found from the llama sample.

Cryptosporidium of the outbreak subtype (BGP16) was identified in the sample collected from one of the petting farm sheep. The specimen could not be identified to the species level (although subtyping indicated it was likely *C. parvum*). *Cryptosporidium* was also found in the donkey sample by microscopy using acid fast stain. The sample was negative by PCR and thus could not be further characterized.

Yersinia enterocolitica was identified in a sample collected from a llama, and *Aeromonas caviae* was identified in a goat sample. Two goats and a donkey tested negative for all pathogens.

Environmental evaluation

Environmental samples from material collected from the ground in the calf pen and the ground in the main animal enclosure were both positive for Shiga toxin genes by PCR (*stx1* in calf pen, *stx2* in main pen); however, STEC isolates were not found for either sample. A sample of feed from inside the calf pen was also positive for *stx1* (no isolate found).

A barn building adjacent to the petting farm enclosure is used for processing and storing apples, storing equipment, and pressing cider. Unpasteurized cider from a wooden press was served to the public only as samples during cider pressing demonstrations that took place multiple times per day on October 8, 9, 15, 16, 22, and 23. Samples of cider were served in small disposable paper cups. Orchard staff used tree-picked washed and graded apples for the cider pressing. The same staff were also charged with care of the petting zoo animals, but reported that they did not care for animals on days when they pressed cider. Staff who pressed and served cider during the demonstrations denied recent illness.

Twelve environmental SpongeSicle™ samples were collected inside the apple processing barn from: the wooden apple press used during demonstrations to serve cider to orchard visitors; an employee hand sink; cloth apple picking baskets used by employees; wooden grading crates; the belt of the apple

processing machine; and, the handles of the machines used by the public to dispense food for petting zoo animals. All of these samples were negative for STEC, *Salmonella*, and *Campylobacter*.

Running water is not available at the orchard for visitors to wash hands. Alcohol-based hand sanitizer is available near the petting zoo area, but two patrons reported that the sanitizers were empty for a period of time on October 8. During the November 4 visit, sanitizer dispensers were cracked and visibly dirty. Small signs on the sanitizer stations encouraged guests to use sanitizer after visiting the animals, but no other public health signage was present.

On November 4, the petting zoo animals were removed from the orchard at the request of Washington County EH specialists to mitigate exposure to the animals by patrons during the orchard's last few open days of the season. On December 15, Washington County staff instructed orchard owners to discontinue serving cider from a wooden cider press, and recommended installation of handwashing stations and signage near the petting zoo animals, and separation of duties between animal and food-handling employees.

This was an outbreak of STEC and *Cryptosporidium* infections associated with drinking unpasteurized apple cider at an apple orchard where petting zoo animals were present. Animals in the petting zoo tested positive for the outbreak strain of *Cryptosporidium*, a strain of STEC closely related to the outbreak strain, and other potential human pathogens. The exact mechanism for contamination of the cider was not identified. However, the petting zoo animals were the ultimate source of the pathogens that caused the outbreak, and the most plausible scenario is that workers who cared for the animals served as mechanical vectors for the pathogens from the animals to the cider pressing equipment. Recommendations were provided to the orchard owners to help prevent future illnesses.