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An outbreak of *Escherichia coli* O157:H7 infections and haemolytic uraemic syndrome associated with consumption of unpasteurized apple cider

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SUMMARY

During October 1996, an outbreak of *Escherichia coli* O157:H7 infections among Connecticut residents occurred. An epidemiologic investigation included enhanced surveillance and a case-control study. Clinical isolates of *Escherichia coli* O157:H7 were typed by pulsed-field gel electrophoresis (PFGE). Implicated cider samples were analysed by culture and polymerase chain reaction (PCR). Consumption of implicated cider was associated with illness; (matched odds ratio = undefined, 95% confidence interval = 3·5–infinity). Ultimately, a total of 14 outbreak-associated patients were identified. All isolates analysed by PFGE yielded the outbreak-associated subtype. *Escherichia coli* O157:H7 was not cultured from three cider samples; PCR analysis detected DNA fragments consistent with *Escherichia coli* O157:H7 in one. This outbreak was associated with drinking one brand of unpasteurized apple cider. PFGE subtyping supported the epidemiologic association. PCR analysis detected microbial contaminants in the absence of live organisms. Washing and brushing apples did not prevent cider contamination.

INTRODUCTION

Escherichia coli O157:H7 infection was first described as a human pathogen in 1982 [1]; infections with *E. coli* O157:H7 have become a leading cause of childhood kidney failure due to haemolytic-uraemic syndrome (HUS). Cattle and other ruminants serve as reservoirs of the bacterium, and consumption of foods of bovine origin have been most closely associated with illness [2–5]. Recently fruit and vegetable products, including apple juice and cider, have been identified as vehicles for infection [6–10].

In October 1996, the Connecticut Department of Public Health (DPH) responded to the report of an unusual number of *E. coli* O157:H7 infections among residents of New Haven County. This report summarizes the investigation of the source of these infections, and reveals an association between illness and a history of drinking a single brand of unpasteurized apple cider.

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METHODS

Case ascertainment

A case was defined as a Connecticut resident with laboratory-confirmed *E. coli* O157:H7 infection or HUS, with onset of illness during 1–11 October 1996. Personnel from the DPH initiated active case finding on 15 October, by notifying all clinical laboratories in Connecticut of the increase in the number of reported cases, and asking them to report suspected cases of *E. coli* O157 to the DPH. We requested that all suspect isolates should be forwarded to the Connecticut Department of Public Health Laboratories (DPHL) for confirmation and determination of H7 antigen status.

Laboratory investigation

Patient stool samples were cultured on sorbitol MacConkey agar at private clinical or hospital laboratories. Isolates of *E. coli* O157 were sent to the DPHL and confirmed with a latex test (Remel, Inc., Lenexa, Kansas) for presumptive identification of O antigen and with antisera for H7 antigen determination (Difco Labs, Detroit, Michigan). Confirmed isolates were sent to the Centers for Disease Control and Prevention (CDC) for pulsed-field gel electrophoresis (PFGE) subtyping using the restriction enzyme *Xba*I (Boehringer Mannheim, Indianapolis, Indiana) as previously described [11]. Strains were also restricted with a different restriction enzyme, *Bln*I (Boehringer Mannheim, Indianapolis, Indiana), to confirm the similarity of patterns. Patients were asked to provide cider samples for microbial analysis. Cider samples were cultured at the DPHL and forwarded to CDC for culture and analysis by polymerase chain reaction (PCR) [12, 13].

Case-control study

To generate hypotheses about sources of infection, five patients were initially given open-ended interviews by telephone with a focus on exposure to children in diapers, food and water consumption, attendance at restaurants and recreational events in the 7 days before onset of illness. Results from these interviews were used to formulate a standardized questionnaire that was used to interview all patients and controls on 17 October. The standardized questionnaire included questions about health history, exposure to meats, cider, apples and other produce items, recreational and drinking waters, children in diapers, and recreational events such as attendance at parties, restaurants, and fairs. The goal was to identify and interview three age-group and sex-matched controls for each case. Controls were selected by progressive, sequential digit dialing from residential telephone exchange lists representing each patient's community. Controls were queried retrospectively about exposures during the same time period as their matched patient. Controls were excluded if they had experienced a diarrhoeal illness during the 20-day period beginning 10 days before the matched patient's onset of illness.

Data analysis

Case-control data were analysed by using EpiInfo version 3.0 (USD, Inc., Stone Mountain, Georgia). Matched odds ratios were calculated for each exposure; a matched odds ratio was considered statistically significant if the 95% confidence interval did not include 1·0. Probability values at the $\alpha = 0{\cdot}05$ level were calculated on the basis of exact number of case and control subjects in each matched set by using EXACT software [14].

Environmental investigation

Personnel from the Connecticut Department of Consumer Protection inspect cider mills annually. The mill that produced Brand A cider was visited on 17 September 1996 as part of a routine inspection, and then on 16, 18, 21 and 22 October as part of the outbreak investigation. Mill personnel were interviewed about cider production methods and procedures. Sources of apples and water were investigated; apple invoices were requested. Equipment used to wash, brush and press apples, and to store and bottle cider were examined.

RESULTS

Case finding

By 17 October, 8 patients were identified and included in the case-control study. Onset of illness occurred during 3–11 October 1996. Of these 8 patients, 6 (75%) were female. The median age was 16 years (range 2–73 years) and they all resided in New Haven County. All 8 patients reported bloody diarrhoea and abdominal cramping: 5 (63%) reported vomiting, 4

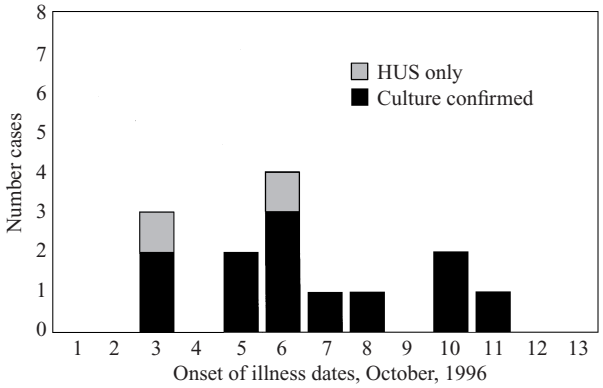

Fig. 1. Epidemiologic curve of *Escherichia coli* O157:H7 infections and haemolytic-uraemic syndrome (HUS) associated with 'Brand A' apple cider, Connecticut, USA, October 1996.

(50%) reported fever, and 2 (25%) developed HUS. The median duration of diarrhoeal illness was 7 days (range: 3–11 days).

Ultimately, a total of 14 patients were identified (although only the first 8 were included in the case-control study); 12 patients had culture-confirmed infection, an additional 2 were diagnosed with HUS, but with no cultures available. Onset of illness occurred during 3–11 October (Fig. 1). Patients not included in the case-control study were questioned about Brand A cider exposure after a recall was initiated. Thirteen of 14 patients reported drinking Brand A cider 3–11 days (median of 7 days) before onset of illness. The remaining individual reported having Brand A cider in the house, but could not remember drinking it. Of these 14 patients, 10 were hospitalized, and 5 developed HUS. Patients resided in 3 of Connecticut's 8 counties; 12 resided in New Haven County. Six patients reported definitive Brand A cider purchase dates during 28 September to 5 October 1996.

Laboratory investigation

Of the 12 patients with stool cultures positive for *E. coli* O157:H7, 10 were analysed by PFGE subtyping at CDC; all 10 were determined to be the outbreak-associated subtype. Three patients provided samples of Brand A cider to the DPHL, all from containers purchased before their onset of illness; no sample yielded *E. coli* O157:H7 upon culture. Of the three cider samples, two were received at CDC and cultured: neither sample yielded *E. coli* O157:H7. One sample provided sufficient material for PCR analysis; PCR generated amplicons consistent with the target genes uidA (252 bp) and stx1 (475 bp), but did not amplify the targeted region of the stx2 gene.

Case-control study

The 8 patients initially identified, and 21 matched controls were included in the case-control study. Consumption of apple cider in the 7 days prior to onset of illness was associated with illness; (matched odds ratio [MOR] = 12·0, 95% confidence interval [CI] = 1·3–111·3, $P = 0{\cdot}002$). Specifically, consumption of Brand A apple cider was associated with illness (MOR = undefined, 95% CI = 3·5–infinity; $P < 0{\cdot}0006$). No other food items or exposures were statistically associated with illness (Table 1).

Environmental investigation

The implicated cider was produced at a mill located in the back of a small retail outlet that sells convenience groceries and seasonal produce. The building is located in a lightly settled area, with no evidence of animal agriculture nearby. The mill had been in seasonal operation from 1921, but was closed 17 October 1996 amid concerns about potentially contaminated cider. A press release and product recall were issued on 18 October, warning consumers of the risk of illness associated with consumption of Brand A cider.

Cider was pressed in a partly enclosed cider shed behind the store. The back of the shed was open to allow access to apples stored outdoors in an uncovered wooden bin. All apples were washed and brushed in a flow-through water system that used potable municipal water. Apples were ground, then funneled into a wooden press. The filter portion of the press was made of metal screens layered with cotton cheesecloth. During the pressing season, the press was disassembled and rinsed nightly with municipal water. Freshly pressed cider was directed into one of two refrigerated stainless steel tanks. Potassium sorbate 0·1%, an antifungal agent, was added to cider before it was bottled in plastic jugs. The cider was not pasteurized.

There were no lot numbers or dates on cider bottles to identify those bottles produced during the dates implicated in the investigation. Apples were obtained from multiple sources. Few invoices were available for review, but a traceback investigation identified at least eight orchards that had provided the majority of the fruit used by the mill. Small numbers of apples may

Table 1. *Results of* E. coli *O157:H7 case-control study analysis for selected exposures, Connecticut, USA, 1996*

Food item	Cases ($n = 8$) Exposed (%)	Controls ($n = 21$) Exposed (%)	Matched OR	95% CI
Any apple cider	7 (88)	5 (24)	12·00	1·3–111·3*
Brand 'A' cider	7 (88)	1 (5)	Undefined	3·5–infinity*
Fresh apples	5 (63)	13 (62)	0·93	0·11–8·42
Lettuce	4 (50)	11 (52)	1·00	0·01–78·5
Hamburger	3 (38)	10 (48)	1·12	0·12–10·33

* $P \leqslant 0{\cdot}002$.

have been obtained from additional orchards because multiple apple brokers provided apples for the mill. First grade and 'drop' apples (fruit gathered from the ground) were used in the production of the cider.

DISCUSSION

This outbreak of *E. coli* O157:H7 infections was associated with one brand of unpasteurized apple cider. A total of 14 patients were identified; 13 had a history of consumption of cider manufactured by the implicated producer. Molecular subtyping of patient isolates supported the epidemiologic association.

Apple cider is a traditional seasonal beverage in New England and is typically prepared by mechanically pressing low-grade and drop apples that cannot be marketed as fresh eating apples. It is a common practice to mix fruit from different growers to obtain a balance of sweet and tart varieties for optimum cider flavor and aroma. Historically in Connecticut, most cider purchased from cider mills and roadside produce stands has been unpasteurized (Connecticut Department of Consumer Protection, personal communication, 12 July 1999).

Apple cider may potentially become contaminated at multiple points during production. In the orchard, apples may come into contact with faecal material on the ground (drop apples), via animal vectors, or via contaminated water used for irrigation or pesticide application. During harvest, faecal material on the hands of workers or on equipment may contaminate apples. Once a wooden press or storage tank becomes contaminated with *E. coli* O157:H7, daily rinsing with potable water may not be sufficient to disinfect the equipment. In this manner, contamination may be perpetuated over several days of cider production.

Previous outbreaks of *E. coli* O157:H7 infections and HUS associated with apple cider have been described [8, 9]. After cider was recognized as a vehicle for *E. coli* O157:H7, some states in the northeast region of the United States (US) formulated regulations or recommendations designed to decrease the risk of cider contamination by enteric pathogens. Recommendations have included: avoiding the use of cow manure to fertilize orchards, only using apples picked from the tree, washing and brushing apples in potable water before pressing, and using preservatives such as sodium benzoate to limit bacterial survival in the finished product.

The cider mill implicated in this outbreak did wash and brush apples in potable water, but drop apples were pressed to make cider. Potassium sorbate 0·1%, an antifungal agent, was used as a preservative, but has been shown to be ineffective in diminishing the survival time of *E. coli* O157:H7 [15]. It is unknown if apples originated in orchards where dropped fruit may have contacted cow or deer manure, or if workers may have been the source of contamination. Because *E. coli* O157:H7 is infectious at low doses, inapparent amounts of faecal contamination on or in apples may have been sufficient to cause an outbreak [4].

Recently, the US Food and Drug Administration has required warning labels be displayed or placed on all containers of unpasteurized apple juice or cider. The labels state: 'WARNING: This product has not been pasteurized and, therefore, may contain harmful bacteria that can cause serious illness in children, the elderly, and persons with weakened immune systems'. This labeling requirement is considered an interim measure until a Hazard Analysis of Critical Control Points (HACCP) plan is phased in for all raw juice producers in the US [16, 17]. While these warning labels are intended to notify individuals in certain high risk groups, other individuals may be at risk from infection by contaminated juice; of the eight patients included in the case-control study, five

patients (63%) were between the ages of 5 and 50 years (9, 13, 18, 34 and 44 years of age).

E. coli O157:H7 was not cultured from any of the three Brand A cider samples submitted by patients. However, all samples were cultured more than 20 days after the cider was purchased, and Zhao reported survival of *E. coli* O157:H7 in apple cider only up to 20 days at 8 °C in the presence of potassium sorbate 0·1% [15]. PCR analysis detected DNA fragments consistent with *E. coli* O157:H7 within the only implicated cider sample tested by this method. The PCR results strongly suggest the presence of the *E. coli* O157:H7 in the juice sample based on detection of the *E. coli* O157:H7-specific uidA allele and the stx1 gene. Although the outbreak strain produced both Shiga toxins 1 and 2, the presence of the stx2 gene was not detected by PCR. Possible explanations for this observation include: the strain in the juice had lost the stx2 gene before testing, the PCR conditions for stx2 were less robust than for the other genes and thus did not amplify a product under these conditions, or the sample may have contained a different strain of *E. coli* O157:H7.

The producer of Brand A cider did not use production codes and did not maintain comprehensive invoices of source apples; this severely limited our ability to trace apples back to specific orchards and to selectively recall product. During this investigation, all Brand A cider had to be recalled due to uncertainty about production dates.

Recommendations to reduce the risk of future cider-associated outbreaks include: development of a cider-specific HACCP plan, producer education, improved sanitary conditions, and the consistent application of good manufacturing practices within cider mills. Although these measures may help to reduce the risk of contamination during apple cider production, bacteriologic quality of the finished cider can vary greatly among producers [18]. At present, only the proper application of pasteurization or other bacteriocidal treatments have the potential to reduce the risk of infection to the consumer [19].

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