Escherichia coli O157:H7 Infections Associated With Consumption of Locally Grown Strawberries Contaminated by Deer

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(See the Editorial Commentary by Cody on pages 1135–7.)

Background. An outbreak of Escherichia coli O157:H7 was identified in Oregon through an increase in Shiga toxin–producing E. coli cases with an indistinguishable, novel pulsed-field gel electrophoresis (PFGE) subtyping pattern.

Methods. We defined confirmed cases as persons from whom E. coli O157:H7 with the outbreak PFGE pattern was cultured during July–August 2011, and presumptive cases as persons having a household relationship with a case testing positive for E. coli O157:H7 and coincident diarrheal illness. We conducted an investigation that included structured hypothesis-generating interviews, a matched case-control study, and environmental and traceback investigations.

Results. We identified 15 cases. Six cases were hospitalized, including 4 with hemolytic uremic syndrome (HUS). Two cases with HUS died. Illness was significantly associated with strawberry consumption from roadside stands or farmers’ markets (matched odds ratio, 19.6; 95% confidence interval, 2.9–∞). A single farm was identified as the source of contaminated strawberries. Ten of 111 (9%) initial environmental samples from farm A were positive for E. coli O157:H7. All samples testing positive for E. coli O157:H7 contained deer feces, and 5 tested farm fields had ≥1 sample positive with the outbreak PFGE pattern.

Conclusions. The investigation identified fresh strawberries as a novel vehicle for E. coli O157:H7 infection, implicated deer feces as the source of contamination, and highlights problems concerning produce contamination by wildlife and regulatory exemptions for locally grown produce. A comprehensive hypothesis-generating questionnaire enabled rapid identification of the implicated product. Good agricultural practices are key barriers to wildlife fecal contamination of produce.

Keywords. Escherichia coli O157; STEC; foodborne disease outbreak; Fragaria; deer.

Escherichia coli O157:H7 infections can range in severity from asymptomatic carriage to bloody diarrhea and severe abdominal cramping, hemolytic uremic syndrome (HUS), and death [1]. The principal reservoirs of E. coli O157:H7 are ruminant animals (eg, cattle, sheep, goats, deer). Infections are acquired by consumption of fecally contaminated water or food (especially meat or produce), via person-to-person spread, and from contact with colonized animals or their environments [1].

During early August 2011, we noted an increase in routinely reported Shiga toxin–producing E. coli (STEC) and E. coli O157:H7 infections among certain northwestern Oregon counties. On 2 August, pulsed-field gel electrophoresis (PFGE) subtyping identified multiple indistinguishable E. coli O157:H7 isolates with a novel pattern. The ensuing investigation—intended to determine...
outbreak extent, source, and best control measures—identified a novel vehicle for *E. coli* O157:H7 infections, and highlights problems concerning fecal contamination of produce from wildlife and the safety and regulatory oversight of agricultural production.

**METHODS**

**Routine Reporting and Case Finding**

*Escherichia coli* O157:H7 infections are legally notifiable in Oregon. Private laboratories are required to forward isolates or broth cultures to the state public health laboratory for confirmation and PFGE subtyping. After the outbreak was recognized, we stimulated reporting by alerting other public health agencies in Oregon and neighboring states. We queried the national PulseNet database for indistinguishable PFGE patterns elsewhere in the country [2].

**Case Definitions**

As a working case definition for our initial analytic studies, we included any *E. coli* O157–infected person from northwest Oregon with illness onset in July or August 2011 and either PFGE pattern EXHX01.1316/ EXHA26.1042 or no PFGE result yet. For the final outbreak summary, we defined confirmed cases as persons from whom *E. coli* O157:H7 with the outbreak PFGE pattern was cultured. Household members of confirmed cases with coincident diarrheal disease were counted as presumptive cases.

**Case Interviews**

As part of routine public health surveillance, cases (or household proxies) were initially interviewed by local health department staff using routine enteric case investigation questionnaires that included 18 exposure questions. During the ensuing outbreak investigation, investigators (re)interviewed cases (or household proxies) with a standard 450-item hypothesis-generating ("shotgun") questionnaire developed in Oregon that includes shopping and eating venues, specific foods consumed, and other potential exposures. Foods of interest were identified by comparing food consumption frequencies among cases with estimated background consumption rates [3] using binomial probabilities. As necessary, cases were recontacted to determine additional details about specific items, including dates and locations of purchase.

**Case-Control Study**

On 5 and 6 August 2011, we conducted a case-control study focused on food exposures flagged by the hypothesis-generating interviews. Each case was matched with 3 control subjects on the basis of neighborhood, sex, and age group (2–17, 18–59, ≥60 years). Control subjects were identified through an online reverse directory [4]. Matched odds ratios were calculated using exact methods (SAS version 9.1, SAS Institute, Cary, North Carolina).

**Product Traceback and Trace Forward**

After the vehicle was identified, we visited points of sale named by cases. Vendors provided information about their suppliers for sales dates in question. After the source farm (farm A) was identified, investigators obtained a list of the farm’s consignees, and traced these consignees forward to identify additional points of sale.

**Environmental Sampling**

We collected leftover frozen and fresh product from farm A, consignees, and case and consumer households. We collected a convenience sample of soil, plant fragments, and deer fecal pellets (fresh and dry) from the ground and irrigation pond water from farm A fields where strawberries had been harvested during the period of case exposure. We estimated the density of deer droppings in farm fields by walking crop rows, counting the number of readily visible discrete dropping piles, and dividing by the area of the field.

**Microbiology**

Environmental samples, including food, were assayed for STEC by multiplex polymerase chain reaction (PCR), using STEC-specific (*eae, stx1, stx2*) and *E. coli* O157:H7-specific (*rfb*) primers [5]. PCR-positive samples were cultured in parallel with and without immunomagnetic separation. *Escherichia coli* O157:H7 isolates were subtyped by PFGE [6].

**RESULTS**

**Descriptive Epidemiology**

We identified 15 cases; 14 were laboratory-confirmed. Illness onsets ranged from 10 July to 28 July 2011 (Figure 1A). All cases were residents of 5 counties in northwestern Oregon. Eleven cases (73%) were female. Median age was 68 years (range, 4–85 years); 3 cases were ≤18 years, 4 were between 19 and 64 years, and 8 were ≥65 years. Thirteen cases reported bloody diarrhea. Six cases were hospitalized, including 4 with HUS. HUS occurred at the extremes of age (<5 and ≥65 years). Two cases with HUS died; both were >75 years of age.

**Patient Interviews**

No noteworthy common exposures were identified from the initial 18-item routine questionnaire interviews. However, by 5 August, hypothesis-generating questionnaire results were notable for strawberry consumption and for shopping at roadside stands or farmers’ markets—both reported by 12 of 13 interviewed cases (92%). Assuming a fresh strawberry consumption rate of 43% within a given week—the rate seen in a
recent population-based Oregon survey [3]—the binomial probability of at least 12 of 13 persons reporting consumption by chance alone was 0.0003. Other foods consumed by cases at a greater than expected frequency included blueberries, raspberries, and cantaloupe.

**Case-Control Study**

The initial case-control data analyzed on 5–6 August included 6 persons whose PFGE results were still pending. Later laboratory tests showed that only 5 of these matched the outbreak pattern; the discordant person was subsequently excluded. Results of the initial and final analyses were similar (data not shown). In the final analysis, 12 cases were matched to 36 control subjects. Illness was significantly associated with consumption of strawberries from roadside stands or farmers’ markets (matched odds ratio, 19.6; 95% confidence interval, 2.9–∞) specifically, but not with strawberry consumption generally.

**Product Traceback and Trace-Forward Results**

Fourteen of 15 cases recalled eating locally grown strawberries. Some cases reported purchases from multiple vendors, and some vendors reported multiple suppliers, but all 14 cases reported consumption of strawberries from at least 1 vendor supplied by farm A. Three cases consumed only strawberries from vendors supplied exclusively by farm A.

Farm A was a family-operated business that during 2011 farmed 8 strawberry fields totaling approximately 14 hectares. Strawberries sold and consumed during the exposure period of cases were harvested from 5 of the 8 fields. Certain fields were contiguous; others were separated by up to 5 km. Conventional (ie, nonorganic) methods were used; manure was not applied. Some but not all fields were irrigated, and those that were irrigated did not all use the same water source. None of the strawberry fields adjoined livestock grazing areas, and there were no confined or concentrated animal feeding operations within 2 km of any of the fields. Fields were harvested that year from late May until 29 July. Berries were hand-picked and boxed in the fields without washing; consignees picked up strawberries at the farm and typically sold most or all of them that same day. No berries were sold to processors. According to the farmer, no illnesses were reported by harvest workers, who had dispersed and were unavailable for interview.

We identified 56 points of sale (farmers’ markets, roadside stands, and farm produce markets) for farm A strawberries, comprising 54 locations in Oregon and 2 in Washington. Strawberries were sometimes resold or bartered multiple times through largely undocumented exchanges between vendors and consignees. Strawberries were retailed in paper cartons without labeling or packaging to indicate farm A.

**Food and Environmental Testing**

On 6 August, we collected a convenience sample of 111 environmental specimens from 5 farm A fields harvested during the likely period of case exposure. By PCR, 10 samples (9%) were positive for *E. coli* O157, and 13 (11.7%) were positive for other STEC. All 10 *E. coli* O157 PCR-positive samples were subsequently culture confirmed for *E. coli* O157. Of the 50 samples that contained visible deer pellets, 10 (20%) were positive for *E. coli* O157. All *E. coli* O157 isolates were indistinguishable from the outbreak pattern by PFGE. Matching isolates were
recovered from each of the fields sampled. Of the 13 samples that were PCR positive for non-O157 STEC, 7 (53.8%) were culture confirmed, including 4 of 50 (8%) that contained visible deer pellets. Non-O157 serogroups were not determined.

On 26 August, we collected 345 deer pellet samples from all 8 farm A strawberry fields that had been in production during 2011. On this occasion, only 6 samples (1.7%) were PCR positive for E. coli O157:H7, including 4 (1.2%) that were culture confirmed. All isolates were indistinguishable from the outbreak pattern by PFGE. Twenty-one other deer pellet samples (6.1%) were PCR positive for non–E. coli O157:H7 STEC, of which 6 (1.7%) were culture confirmed. Three samples of irrigation pond water were negative for STEC.

Deer fecal pellet piles were readily visible in all fields during both visits, and black-tailed deer (Odocoileus hemionus) were observed feeding in 1 field during our 6 August visit (Figures 2 and 3). On 26 August, we counted a median of 45 deer fecal pellet piles per hectare (range, 0.8–96 piles per hectare) in the 8 fields.

One of 4 frozen strawberry samples initially tested PCR positive for stx2, but none was culture confirmed for STEC.

Microbiology
All outbreak-associated E. coli O157:H7 isolates shared an indistinguishable PFGE pattern combination (XbaI, EXHX01.1316; Bln1, EXHA26.1042) that was otherwise novel to the PulseNet national database. Isolates were PCR positive for stx2.

Public Health Measures
On 6–7 August, we provided a summary of our findings to the owner of farm A, other public health agencies, and interested business groups. On 8 August, in cooperation with the owner of farm A, we announced our findings and advised the public to discard any remaining fresh or frozen strawberries that might have come from farm A or any of the identified retail outlets.

Postscript
Several months after the outbreak ended, a PFGE-matching E. coli O157:H7 isolate was cultured from an Oregon child with HUS. The child lived <1 km from several farm A fields. Although the child had not eaten strawberries from farm A, the parents reported that deer were frequently observed in their yard and garden. Deer tracks were observed within 30 m of the house, but no fecal pellets were found for testing. We cultured E. coli O157: H7 from a single aliquot of dirt and debris taken from the household vacuum cleaner bag. By PFGE, the vacuum cleaner isolate was indistinguishable from the outbreak (and child’s) isolates.

DISCUSSION
This outbreak was caused by consumption of strawberries contaminated with deer feces. The strawberries were locally grown and retailed at small and mostly unregulated venues. Although this investigation did not include a comprehensive ecological survey, potential sources other than deer (eg, nearby livestock, irrigation water, processing, or farm workers) could be reasonably excluded as causes of contamination. Deer feces with the outbreak strain were found in multiple locations over an area large enough to suggest that multiple deer were colonized.

Neither strawberries nor any other berries have previously been identified as a vehicle for E. coli O157:H7 transmission. Lettuce, spinach, and other ground crops are well-documented sources [7–9]. Strawberries have been previously identified as vehicles for hepatitis A [10, 11] and norovirus [12]—both viruses with human reservoirs.

Deer were first identified as a source of E. coli O157 infections following a 1995 outbreak that occurred <100 km from farm A [13]. Handling or consuming deer or elk meat has been repeatedly identified as a source of E. coli O157:H7 infections [13–15].
Deer or elk were considered the likeliest sources for several outbreaks caused by consumption of contaminated apple cider and drinking water [13, 16–18]. Colonized large game mammals, including feral swine, may also have contributed to the contamination of spinach fields implicated in a large E. coli O157 outbreak in 2006 [19].

We found extensive evidence of deer and E. coli O157:H7 in farm A’s fields. On our initial survey, 20% of samples containing visible deer pellets were culture positive for E. coli O157; 20 days later the observed prevalence was 1.2%. Although we note that summer temperatures had increased considerably during the interim, the explanation for the observed decline is uncertain. In previous studies, the prevalence of E. coli O157 among deer fecal samples or sampled from individual deer has varied. Two small outbreak-associated surveys of deer pellets in Oregon and California yielded 9% and 10% positive test results, respectively [13, 17]. Studies not associated with outbreak investigations have reported lower prevalence of E. coli O157:H7 in wild deer or deer fecal pellets, ranging from 0% to 2.4% [20–25]. Non-O157 STEC has also been found in deer and venison [26–28], and at least 1 outbreak of these infections among humans was traced to venison [29].

Deer might be more relevant to the contamination of produce and the transmission of E. coli O157:H7 and human illness than is widely appreciated. Deer are highly mobile and share their environment with humans, livestock, other wildlife, and croplands. Deer are common in many agricultural areas, making risk mitigation difficult if strategies depend solely on restricting access; efforts to separate them completely from agricultural lands where they reside would be impractical and costly. The relative contributions of animal density, diet, weather, seasonality, proximity to livestock, and other factors to the observed prevalence of E. coli O157:H7 among wild cervids and the risk of crop contamination are unknown [30]. Although deer feces were readily apparent in farm A’s fields in 2011, we could not assess whether they were above or below historical averages. In the most highly contaminated farm A field, the density of fecal piles was approximately 1/100 m². Other farmers and agricultural experts in Oregon and California opined that the fecal contamination levels at farm A may have been exceptional, but we were unable to collect sufficient data for comparison.

This outbreak highlights problems concerning fecal contamination of produce from wildlife and the safety and regulatory oversight of agricultural production. Farming and processing practices are the most important barriers to contamination, because even the use of sanitizing solutions or vigorous washing—which is relatively infeasible with strawberries or other berries—is unlikely to eliminate bacterial surface contamination [31]. In 1998, the US Department of Agriculture and the Food and Drug Administration (FDA) published the Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables [32], which outlined relevant best practices. In 2005, the California Strawberry Commission published food safety recommendations specific for strawberry growers [33] based on good agricultural practices (GAPs). GAPs for produce growers include water quality, worker health, field sanitation, postharvest sanitation, and record keeping. All of these are relevant to strawberry growers, but field sanitation was directly related to produce contamination in this outbreak. Observing a no-harvest zone around obvious fecal deposits or restricting harvests from heavily contaminated fields, as recommended by the California Strawberry Commission guidelines, would presumably reduce risk [33]. Had the California guidelines been applied at farm A, no-harvest zones would have resulted, although the degree of fecal contamination might have led some entire fields to be abandoned.

GAP certification is increasingly required of farmers by large commercial customers. In contrast, smaller-scale farmers whose products may be sold at local farmers’ markets, on-farm stands, and roadside stands have fewer incentives to seek certification. Farm A was not GAP-certified. Moreover, small farm operations with limited geographic product distribution, including farm A, are exempted from proposed US federal regulatory oversight under the 2011 FDA Food Safety Modernization Act [34].

From 2000 to 2012, the number of registered farmers’ markets in the United States increased from 2863 to 7864 [35], suggesting that this is an increasingly popular source of food for US consumers. There is little reason to believe that small farms are inherently safer than larger ones when it comes to risk of microbial contamination. Food producers of all sizes should adopt practices that reduce consumer risk. Collaborative efforts are under way to make GAPs achievable even for small-scale produce farmers and packers [36].

The use of a comprehensive hypothesis-generating questionnaire enabled us to identify strawberries as a previously unsuspected vehicle within days of the initial reports of matching PFGE cases (Figure 1B). Both strawberry consumption and shopping at roadside stands and farmers’ markets were flagged in the interviews. These findings were quickly corroborated by a case-control study and traceback. The comprehensive hypothesis-generating questionnaire has proven useful in resolving previous national outbreaks caused by spinach (E. coli O157:H7) and almonds (Salmonella enterica). Widespread use of such questionnaires derived from a library of standardized exposure elements would improve the ability of public health investigators to readily pool data from multiple jurisdictions and questionnaires. Outbreaks associated with commercially distributed foods are likely to continue, and rapid identification of vehicles enables public health action that can minimize morbidity and mortality as well as collateral economic damage.

Notes

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References