# A Cluster of *Escherichia coli* O157: Nonmotile Infections Associated with Recreational Exposure to Lake Water

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## **SYNOPSIS**

**Objectives.** To identify cases and determine risk factors for an outbreak of *Escherichia coli* (*E. coli*) O157:nonmotile (NM) infections in children attending a summer day care program in California.

**Methods.** The authors conducted a retrospective cohort study; the cohort comprised first and second graders who attended the day care program during the last week in August 1999. Shiga toxin testing and molecular subtyping using pulsed-field gel electrophoresis were performed on isolates. Lake water, lake bottom sediment samples, and waterfowl feces from the lake environs were cultured for *E. coli* O157.

**Results.** Three cases of Shiga toxin-producing *E. coli* O157:NM infections with matching pulsed-field gel electrophoresis patterns and four probable cases were found. Children who swallowed more than a mouthful of water had a higher attack rate than those who swallowed less than a mouthful or none at all (43% vs. 10%, relative risk = 4.43, 95% confidence interval 1.12, 17.50).

**Conclusions.** *E. coli* O157:NM infections were associated with swallowing water from a freshwater lake. Potential sources of contamination include feces from humans, cattle, or deer. This outbreak illustrates the value in screening patients with diarrhea for *E. coli* O157, submitting isolates to public health laboratories, and using molecular techniques to identify related cases. Outbreaks associated with contaminated freshwater could be averted by prevention and early detection of contamination.

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#### INTRODUCTION

Escherichia coli (E. coli) O157 is a well-recognized cause of watery diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome. Reported vehicles for E. coli O157 infection include ground beef, unpasteurized juice, and sprouts. This article concerns an outbreak of Shiga toxin-producing E. coli O157:nonmotile (NM) infections associated with recreational exposure to freshwater.

In California, *E. coli* O157:H7 infections are reportable. Cases go undetected because diarrheal illness is commonly treated symptomatically, and stool cultures are not performed. In general, a special request must be made to culture for *E. coli* O157. If *E. coli* O157 is cultured, the laboratory can forward the isolate to the California Department of Health Services/Microbial Diseases Laboratory (MDL) for Shiga toxin testing and molecular subtyping using pulsed-field gel electrophoresis (PFGE). PFGE subtyping allows for recognition of a subset of *E. coli* O157 cases that may have a possible common source.<sup>4</sup>

On September 24, 1999, MDL identified three cases of Shiga toxin-producing *E. coli* O157:NM infections with matching PFGE patterns in children, with onsets from August 29–31. Exploratory interviews with the parents of the children revealed that, in the week before their onsets, all three patients attended the same day care program in California. Two patients were entering the first grade and one was entering the second grade. The day care program did not routinely provide meals to the students; however, in the week before the onsets, the day care sponsored two events with meals. On Wednesday, August 25, there was a swimming trip to a local lake with a hot dog lunch, and on Friday, August 27, there was a pizza lunch.

### **METHODS**

We defined a "confirmed case" as a laboratory-confirmed case of Shiga toxin-producing *E. coli* O157:NM with a matching PFGE pattern in a child who attended the day care program, with onset of diarrhea (defined as at least three loose stools in 24 hours) between August 26 and September 6. We defined a "probable case" as a case of diarrheal illness with onset between August 26 and September 6 in a child who attended the day care program. To find additional cases during the exploratory phase of the investigation, investigators asked the physicians who diagnosed the three confirmed cases about other cases of diarrheal illness seen at their practices during August and September.

We conducted a cohort study on a convenience sample of first and second graders who attended the day care program the last week in August to determine risk factors for illness and identify additional cases. To identify this cohort, the day care program compiled lists of all children entering the first and second grades who attended the summer program. The sample comprised all children whose parents could be reached by telephone. Interviews were conducted from mid- to late-September. We queried about food exposures at the two lunches, exposures to lake water during the swimming trip, and symptoms associated with any diarrheal illness between August 26 and September 6.

## Statistical analysis

Probable cases were not distinguished from confirmed cases in the analysis. We calculated relative risks, 95% confidence intervals, and Mantel-Haenszel p-values using EpiInfo 6.0.<sup>5</sup>

## Laboratory and environmental investigations

We tested all isolates for Shiga toxins using a verocell assay<sup>6</sup> and subtyped the isolates using PFGE.<sup>4</sup> The local public health laboratory cultured lake water, lake bottom sediment samples, and waterfowl feces from the lake environs for *E. coli* O157. Environmental samples were collected eight weeks after the outbreak occurred.

We observed the lake's features, wildlife, and water sources, investigated the possible sources of contamination, and reviewed the city's weekly records of lake coliform counts for the five weeks starting August 2.

#### **RESULTS**

We identified three confirmed and four probable cases of Shiga toxin-producing *E. coli* O157:NM infections. The four probable cases were identified through the cohort study. Six case-patients had onsets between August 28 and August 31; the seventh had onset the first week of September, but could not remember the specific date. Two of the confirmed case-patients and none of the probable cases had bloody diarrhea; no patients had hemolytic uremic syndrome. None of the case-patients were hospitalized, and there were no deaths.

Ninety-two children attended the first- and secondgrade summer sessions. Fifty-three interviews were conducted; of these, 43 (81%) children attended the last week of August and were included in the study. Based on these data, we estimate there were about 75 children in the cohort, and the 43 interviewed children constituted approximately 57% of the cohort. All seven case-patients attended the swimming event, whereas only three patients attended the pizza lunch. The Table shows the relative risks for the cohort study. Children who swallowed more than a mouthful of lake water had a higher attack rate than those who swallowed less than a mouthful of lake water or none at all (43% vs. 10%, relative risk [RR] = 4.43, 95% confidence interval [CI] 1.12, 17.50, Mantel-Haenszel *p*-value = 0.03).

#### Laboratory and environmental investigations

All three isolates produced Shiga toxins; two of the clinical isolates had identical PFGE patterns, and the third isolate differed by only one band. The PFGE pattern of the case-patients' isolates was not seen in any of 70 other isolates tested in California from August through October 1999. *E. coli* O157 was not isolated from lake water, lake bottom sediment samples, or waterfowl feces.

The lake at which the swimming event occurred is a freshwater lake supplied by a river (Figure 1), and is open to swimmers from Memorial Day through Labor Day. The lake is drained annually on November 1 and

filled in the spring. The swimming beach is fenced-off from the rest of the park and access is via an entry gate. Restrooms are available within the fenced-off area. Boating is allowed on the lake, and there is access to the river. At the time of the outbreak, there was a chlorinated kiddie pool within the fenced-off beach area; however, diapered children were also allowed to use the lake.

Numerous ducks and geese were observed on the lake and on the adjacent beach and lawns. A herd of cattle was visible on the opposite bank of the river. No deer were sighted; however, the park manager reported numerous deer in the area.

The review of the lake's coliform counts revealed a spike in total and fecal coliform counts two weeks before the swimming trip (Figure 2).

## **DISCUSSION**

Our investigation revealed an association between swallowing more than a mouthful of lake water and Shiga toxin-producing *E. coli* O157:NM infections. There are several possible sources of contamination: an uniden-

Table. Cohort study results, outbreak of E. coli O157:NM, California, 1999

Exposure	III among number exposed (Percent)	III among number exposed (Percent)	Relative risk <sup>a</sup>	95% Confidence interval <sup>b</sup>	M-H p-value°
Hot dog at lake <sup>d</sup>	5/34 (15)	1/4 (25)	0.59	0.09, 3.86	0.60
Water brought					
from home	4/17 (24)	1/18 (6)	4.24	0.52, 34.20	0.13
Water from the drinking					
fountain at swimming trip	1/2 (50)	3/31 (10)	5.17	0.89, 29.86	0.10
Swallowed lake water					
(vs. not at all)d	4/19 (21)	2/19 (11)	2.00	0.41, 9.65	0.38
Swallowed more than a mouthful of lake water					
(vs. not at all)	3/7 (43)	2/19 (11)	4.07	0.85, 19.48	0.07
Swallowed more than a mouthful of lake water (vs. less than a mouthful					
or not at all) <sup>d</sup>	3/7 (43)	3/31 (10)	4.43	1.12, 17.50	0.03
Attended the pizza party	3/25 (12)	2/16 (12.5)	0.96	0.18, 5.13	0.96

<sup>&</sup>lt;sup>a</sup>Risk ratio.

E. coli = Escherichia coli

NM = nonmotile

b95% confidence interval calculated using Epi Info 6.0.

<sup>&</sup>lt;sup>c</sup>Mantel-Haenszel (M-H) *p*-value is the corrected Mantel-Haenszel summary chi-square *p*-value calculated with Epilnfo 6.0.

<sup>&</sup>lt;sup>d</sup>The parent of one confirmed patient refused to participate in the cohort study; that child attended the swimming trip and did swim in the lake but we had no information on other possible risk factors.

River (flowing towards the lake)

Nature trail

Approx. 0.5 mile

Figure 1. Schematic of the lake and river, and approximate location of the cattle seen upstream of the lake

tified child in the lake with *E. coli* O157 infection; feces of cattle just upstream of the lake; and feces from deer known to frequent the lake environs. Birds have not been shown to be a primary source of *E. coli* O157 infection, but they could have been colonized from a contaminated environment and could have amplified the *E. coli* contamination in the lake.

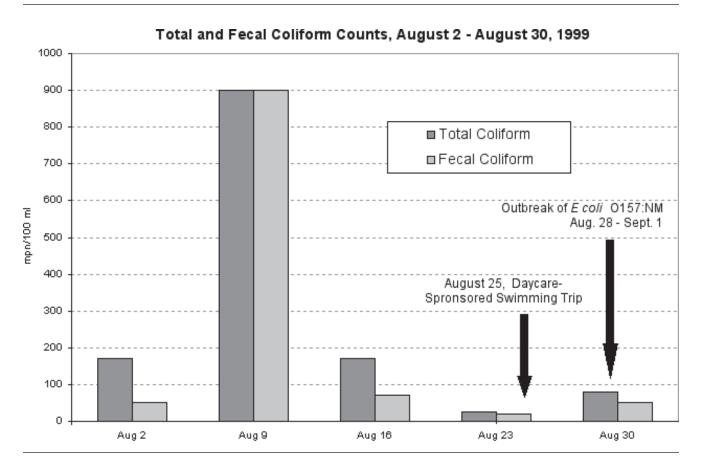
The lake had been closed to bathers for the season on Labor Day, and therefore no additional control measures were necessary to prevent additional cases that season. Our plans to prevent future outbreaks include aggressive water testing protocols, warning signs emphasizing the risks associated with swallowing water, and restriction of diapered children to the chlorinated kiddie pool. Other preventive measures include providing convenient bathrooms with appropriate diaper-changing and handwashing facilities, and displaying signs advising people to wash their hands after using the bathroom.

Transmission of *E. coli* O157 from freshwater lakes and ponds has been documented,<sup>7-11</sup> but is not common. In 1991, 21 cases of *E. coli* O157:H7 associated with swimming in a lake were reported in Oregon.<sup>10</sup> In 1995, five children in Illinois with *E. coli* O157:H7 infection had statistically significant exposure to lake water.<sup>7</sup> In these outbreaks, human contamination of lake water was postulated as the source.

The negative cultures for *E. coli* O157 from lake water and lake bottom sediment samples collected eight weeks after the outbreak do not rule out the possibility that the lake was contaminated during the outbreak period. Wang and Doyle found that *E. coli* O157:H7 can enter a viable, but nonculturable state in water, and demonstrated that the organism can survive in lake water for at least seven weeks at 25°C, and longer at cooler temperatures.<sup>12</sup>

The elevated lake coliform counts two weeks before the outbreak exceeded recommended levels for recre-

Figure 2. Total and fecal coliform counts for the lake from August 2 - August 30, 1999



ational use. Current guidelines recommend that, if a 30-day average fecal coliform count exceeds 200 mpn/100 ml, or a single sample exceeds 400 mpn/100 ml, the lake should be closed to swimmers until the count drops below the cut-off level and a warning should be posted. According to the city, the lake was closed after the elevated count on August 9, but was reopened after the count dropped the following week. It is possible that *E. coli* O157 was a contaminant when the lake was closed, persisted in the lake, and caused the outbreak two weeks later.

There were several possible limitations to this study. There may have been recall bias in interviewing 6- and 7-year-old children, via their parents, one month after exposure. It was difficult to quantify exposures, such as the amount of time in the water and the quantity of water swallowed. We asked about "mouthfuls" of water, believing that a child could more easily comprehend that concept rather than other measures, such as "teaspoonful." Finally, we were unable to collect deer or cattle feces for *E. coli* O157 testing.

Despite the resources available to diagnose and molecularly characterize E. coli O157 infections, few physicians in California request cultures for E. coli O157. In 1999, 201 E. coli O157 cases were reported in California, and 163 isolates were forwarded to the MDL. Mead et al. estimated that, for each reported case of E. coli O157:H7, 13–27 cases go unreported.<sup>13</sup> Using the midpoint of this range as a multiplier, more than 4,000 E. coli O157 cases probably occurred in California in 1999. Because physicians associated with this outbreak requested culturing for E. coli O157, and because isolates were forwarded for PFGE subtyping, this cluster was identified and investigated. Recognition of this outbreak illustrates the value in screening for E. coli O157 in patients with unexplained diarrhea, submitting isolates of E. coli O157 from clinical labs to public health labs, and using molecular techniques to identify related cases. Public health officials should include questions about exposure to recreational freshwater when investigating cases of E. coli O157 infection.

Outbreaks associated with contaminated freshwater

lakes can be prevented through a combined approach of measures to prevent contamination, and then early detection and remediation if contamination occurs. Contamination can be prevented by limiting access of livestock to lakes and upstream rivers where possible; by limiting access of diapered youngsters and providing alternatives, such as a chlorinated kiddie pool; and by providing appropriate diaper-changing and handwashing facilities convenient to the swimming beach. Early detection of contamination would include water sampling in areas where people swim, taking multiple samples at various depths, and sampling on an appropriate schedule. When excessive coliforms are detected, the lake can be closed to prevent exposure to *E. coli* O157.

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