A Nationwide Case-Control Study of *Escherichia coli* O157:H7 Infection in the United States

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Risk factors for *Escherichia coli* O157:H7 infection were investigated in a case-control study at 10 medical centers throughout the United States. Among 73 case-patients and 142 matched controls, exposures in the 7 days before illness associated with *E. coli* O157:H7 infection in univariate analysis included consumption of hamburger (matched odds ratio [MOR], 3.8; 95% confidence interval [CI], 1.9–7.9), undercooked hamburger (MOR, 4.5; 95% CI, 1.6–12.2), or hot dogs (MOR, 2.2; 95% CI, 1.1–4.4); eating at a fast-food restaurant (MOR, 2.3; 95% CI, 1.1–4.6); drinking unchlorinated well water (MOR, 2.4; 95% CI, 1.1–5.7); swimming in a pond (MOR, 5.4; 95% CI, 1.1–26.0); and having a household member with diarrhea (MOR, 11.9; 95% CI, 2.7–53.5). In multivariate analysis, only eating undercooked hamburger remained associated with infection. Seven (8%) of 93 patients developed hemolytic uremic syndrome and 1 died. Prevention strategies aimed at modifying risk factors may help to reduce the risk of infection with *E. coli* O157:H7.

*Escherichia coli* O157:H7 is now recognized as an important cause of bloody diarrhea and the leading cause of postdiarrheal hemolytic-uremic syndrome (HUS) among children in the United States and Canada [1]. Infection with *E. coli* O157:H7 results in a disturbingly high frequency of serious complications. Data from outbreak investigations in the United States suggest that 5%–10% of infected children develop HUS. The mortality rate among children with HUS is 3%–5% [2, 3].

Most information on risk factors associated with *E. coli* O157:H7 infection in the United States has come from outbreak investigations. Among identified dietary risk factors, foods of bovine origin, particularly undercooked ground beef, have been the most frequently implicated source [4–10]. Other foods, such as apple cider and mayonnaise-containing sauces, also have been implicated in outbreaks, although the original source of contamination was often suspected to be of bovine or other animal origin [11, 12]. Investigations of clusters of infections have also documented nondietary risk factors such as person-to-person transmission in day care settings or swimming in contaminated water [13–16].

Surveillance data indicate that most infections due to this organism do not occur in the setting of recognized outbreaks [17, 18]. In addition, by definition, outbreak investigations represent situations that are geographically and temporally circumscribed. We therefore sought to investigate risk factors for sporadic *E. coli* O157:H7 infections by conducting a 2-year case-control study in 10 sites throughout the United States.

Materials and Methods

Case ascertainment. The case-control study was nested in a multicenter investigation of the frequency of isolation of *E. coli* O157:H7 and other bacterial enteric pathogens in clinical settings; methods for the conduct of that study have been described in detail elsewhere [19]. In brief, an announcement of the study and request for participation from interested hospitals was made in a hospital newsletter with national circulation [20]. The hospitals were chosen on the basis of geographic location, willingness to participate, and the expectation that an adequate number of outpatient stool cultures would be performed each year. Ten hospitals were enrolled, at least 1 from each of the four census divisions of the United States. Eight hospitals served a general patient population including all age groups, and 2 served primarily pediatric populations; all served both inpatients and outpatients. At each participating hospital, all specimens studied were fecal samples (rectal swabs or whole stools) from persons of all ages, either inpatient or outpatient; these samples were submitted for routine pathogen determination to the hospital’s clinical microbiology laboratory. The study was conducted from October 1990 through October 1992.

Case-control study. For each patient from whom *E. coli* O157:H7 was isolated, information was obtained from several sources, including hospital charts, laboratory records, and tele-
phone interviews with the patient and health care provider. Hospital charts were reviewed to obtain demographic and clinical information. Laboratory records of fecal specimens yielding *E. coli* O157:H7 were reviewed for information on the date the specimen was obtained and the presence of visible or occult blood. Using standardized questionnaires, we interviewed the patient (or parent or guardian if the patient was unable to answer questions) and health care provider by telephone about symptoms, course of the illness (including the occurrence of complications such as HUS or surgery), medications taken during the illness, and dietary and environmental exposures during the 7 days before diarrhea onset. Patient interviews were conducted a median of 6 days (range, 1–63; 73% within 10 days) after the stool specimen was received in the laboratory. Health care providers were interviewed to obtain information on laboratory test results, therapy administered, and complications. For laboratory test results, such as creatinine or hemoglobin levels, only the most abnormal value for each patient was recorded.

For each patient enrolled, 2 age- and sex-matched controls were identified by the patient’s health care provider from persons seen at the same health care facility for a non-gastrointestinal illness during the week before or after the patient’s visit. Persons were eligible to be controls if they or other members of their household had not had diarrhea in the 7 days preceding the health care facility visit. Controls were matched to patients using the following age groups: 0–5 months, 6–11 months, 1–4 years, 5–9 years, 10–19 years, 20–39 years, 40–59 years, and ≥60 years. Interviews with controls were completed within 4 weeks of the date of visit to the health care facility by the matched patient. Controls were asked about the same exposures as the cases for the 7 days before the interview.

Laboratory. To identify *E. coli* O157:H7, all fecal specimens were plated onto sorbitol-MacConkey agar, and plates were incubated at 37°C for 24 h. Three sorbitol-negative colonies were tested for agglutination with O157 latex reagents (Pro-Lab, Round Rock, TX). The O157-positive colonies were sent to the Centers for Disease Control and Prevention for biochemical identification and serotyping [21]. Isolates confirmed as *E. coli* O157:H7 or O157:NM were tested for production of Shiga toxin (Stx) 1 and 2 (formerly called Shiga-like toxins I and II [22]) and for the presence of stx genes by hybridization with oligonucleotide probes [23].

Definitions. Isolates identified as O157:H7 or O157:NM that produced Stx were considered as *E. coli* O157:H7 strains. Definite HUS was defined as the triad of acute microangiopathic hemolytic anemia, thrombocytopenia, and impaired renal function as determined by the following laboratory criteria: hemoglobin <12 g/dL, evidence of red blood cell fragmentation on a peripheral blood smear, platelet count \(<150,000/\mu\text{L}\), and creatinine level of \(>1.0\) mg/dL for patients <13 years of age or \(\geq 1.5\) mg/dL for those \(\geq 13\) years of age. Probable HUS was defined as having the specific criteria for anemia, thrombocytopenia, and impaired renal function without documented evidence of microangiopathy.

Data analysis. Differences in proportions were analyzed using a \(\chi^2\) or Fisher’s exact test. For normally distributed data, differences in means were compared using Student’s t test; for nonparametric data, differences in medians were compared by the Wilcoxon two-sample test. Conditional logistic regression analysis was performed to assess factors associated with *E. coli* O157:H7 infection; factors found to be significantly associated with infection in single variable conditional logistic regression models were then tested in multiple variable models to assess whether they were independently associated. For the analysis of characteristics associated with HUS, continuous variables were also defined as categorical variables by dividing at upper or lower quartile values. For all statistical tests, a two-tailed \(P\) value <.05 was considered significant.

Results

Case ascertainment. During the 2-year course of the study, fecal specimens from 30,463 persons were examined. *E. coli* O157:H7 was isolated from 118 (0.39%) of the total number of specimens; clinical findings from these 118 patients have been summarized previously [19]. Of the 118 patients from whom *E. coli* O157:H7 was isolated, a case questionnaire was completed for 93 (79%). The median age of the 93 patients was 22 years (range, 4 months to 87 years); cases occurred in all age groups, and 27 (29%) were in persons at least 50 years old. Fifty-one percent of case-patients were male, and 97% were white.

Clinical findings: patients without HUS. Among the 93 patients, 86 (92%) did not develop HUS. Clinical findings among these 86 patients included diarrhea (100%), abdominal cramps (92%), bloody diarrhea (90%), nausea (45%), subjective fever (45%), and vomiting (37%). Among patients with information on the maximum number of stools in a 24-h period, the median number of stools was 16 (range, 2–90). At least 1 day of work or school was missed by 73% of 62 patients; the median number of days missed was 4 (range, 1–30). Forty-five percent of patients were hospitalized. Over 40% of patients without HUS were treated with an antimicrobial agent for their illness, and 20% had sigmoidoscopy or colonoscopy; 4% had a barium enema, and 2 (3%) underwent abdominal surgery (1 for presumed hernia repair, 1 for presumed intussusception). Abnormal hemolytic values and renal function were noted in some patients: 9 (15%) of 59 had a hemoglobin level of <12 g/dL, 4 (7%) of 57 had a platelet count of <150,000 cells/\(\mu\text{L}\), 7 (16%) of 45 had a blood urea nitrogen level of \(>20\) mg/dL, and 9 (18%) of 49 had a creatinine level of >1.0 mg/dL.

Clinical findings: patients with HUS. The 7 (6 definite and 1 probable) patients with HUS ranged in age from 1 to 82 years (median, 7); 4 were male. Patients with HUS had a lower median age (7 years) than those who did not (30 years), although this difference was not statistically significant. One patient with HUS, a 22-month-old girl, died after a 3-month hospitalization complicated by chronic renal failure and stroke.

Four of the 7 patients with HUS received an antimicrobial agent for their illness; sigmoidoscopy or colonoscopy was performed on 1, abdominal surgery on 2 (1 appendectomy, 1 unknown procedure), and none had a barium enema. The median highest blood cell count among HUS patients was 19,050 cells/\(\mu\text{L}\) (range, 11,400–30,200 cells/\(\mu\text{L}\); the median lowest hemoglobin level was 8.2 g/dL (range, 6.9–10.6 g/dL); and the median lowest platelet count was 43,000 cells/\(\mu\text{L}\) (range, 28,000–137,000 cells/\(\mu\text{L}\)). The median highest blood urea nitrogen was 69 mg/dL (range, 34–108 mg/dL) and median highest creatinine level was 2.7 mg/dL (range, 1.9–5.5 mg/dL).
Table 1. Factors associated with \textit{E. coli} O157:H7 diarrhea in univariate analysis, \textit{E. coli} O157:H7 nationwide case-control study.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Patients</th>
<th>Controls</th>
<th>Matched odds ratio (95% confidence interval)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burger</td>
<td>59/71 (83)</td>
<td>76/141 (54)</td>
<td>3.8 (1.9–7.9)</td>
<td>.002</td>
</tr>
<tr>
<td>Burger, pink in middle*</td>
<td>25/53 (47)</td>
<td>12/76 (16)</td>
<td>4.5 (1.6–12.2)</td>
<td>.003</td>
</tr>
<tr>
<td>Hot dog</td>
<td>34/72 (47)</td>
<td>47/142 (33)</td>
<td>2.2 (1.1–4.4)</td>
<td>.03</td>
</tr>
<tr>
<td>Chicken</td>
<td>46/70 (66)</td>
<td>118/140 (84)</td>
<td>0.3 (0.1–0.7)</td>
<td>.002</td>
</tr>
<tr>
<td>Dining, fast-food restaurant</td>
<td>44/73 (60)</td>
<td>64/142 (45)</td>
<td>2.3 (1.1–4.6)</td>
<td>.02</td>
</tr>
<tr>
<td>Water, drank well water</td>
<td>17/54 (32)</td>
<td>18/111 (16)</td>
<td>2.4 (1.1–5.7)</td>
<td>.04</td>
</tr>
<tr>
<td>Recreational, swim in pond</td>
<td>8/72 (11)</td>
<td>7/137 (5)</td>
<td>5.4 (1.1–26.0)</td>
<td>.04</td>
</tr>
<tr>
<td>Person in household with diarrhea</td>
<td>12/73 (16)</td>
<td>2/140 (1)</td>
<td>11.9 (2.7–53.5)</td>
<td>.001</td>
</tr>
</tbody>
</table>

NOTE. Factors not associated with \textit{E. coli} O157:H7 diarrhea included having eaten at delicatessen, cafeteria, or any other type of restaurant other than fast food; meat defrosting techniques; animal contact; prior gastric surgery; day care attendance; or having changed diapers or cared for resident in nursing home or hospital.

* Significantly associated with infection in multivariate conditional logistic regression analysis.

\textbf{Risk factors for \textit{E. coli} O157:H7 infection.} At least 1 age-, sex-, and facility-matched control was located for 73 (79\%) of the 93 patients for whom a case questionnaire was completed; 2 patients were matched with 3 controls, 65 patients with 2 controls, and 6 patients with 1 control, for a total of 142 controls. Patients and controls were enrolled from all sites except Florida and Michigan. Enrolled patients did not differ significantly from those who were not enrolled in median age, gender, or measures of illness severity.

Exposures in the 7 days before illness onset significantly associated with \textit{E. coli} O157:H7 diarrhea in univariate conditional logistic regression analyses are shown in table 1. Other than hamburgers and hot dogs, no other foods were positively associated with illness, including other beef products (steak, veal), other meats or poultry (lamb, ham, pork, or turkey), or dairy products (cheese or unpasteurized milk). Chicken consumption was protective. Other significant risk factors in the 7 days before illness onset in univariate analysis included eating at a fast-food restaurant, having well water as a source of drinking water, swimming in a pond, and having another person in the household with diarrhea.

In a conditional logistic regression model assessing any hamburger consumption and consumption of hamburgers that were undercooked (described as pink in the middle), only eating the undercooked hamburgers remained independently associated with \textit{E. coli} O157:H7 infection. Undercooked hamburger consumption was modeled with the other factors associated with infection in univariate analysis; in these models, none of the other factors remained independently associated with \textit{E. coli} O157:H7 infection.

We also explored risk factors among persons who did not eat undercooked hamburgers (28 patients, 70 controls). In unmatched univariate analysis, these patients were more likely than controls to have eaten steak (14/26 [54\%] patients vs. 23/70 [33\%] controls, \(P = .06\), to have been exposed to a person in the household with diarrhea (4/28 [14\%] patients vs. 1/70 [2\%] controls, \(P = .02\)), and to have drunk well water (8/24 [33\%] patients vs. 7/55 [13\%] controls, \(P = .06\)). However, because of small numbers, in matched analysis these models did not converge.

\textbf{Factors associated with HUS.} We compared the prevalence of various factors among patients with \textit{E. coli} O157:H7 infection with and without HUS. Vomiting within 3 days of diarrhea onset was the only symptom significantly associated with HUS. Overall, treatment with antimicrobial agents within 3 days of diarrhea onset was not associated with HUS. However, among those <13 years old, patients who developed HUS were more likely to have received any antimicrobial agent within 3 days after diarrhea onset than were those who either received an antimicrobial agent more than 3 days after diarrhea onset, or who did not receive any antimicrobial agents (3/6 vs. 1/23; relative risk, 11.5; 95\% confidence interval, 1.4–91.8, \(P = .02\)). Of the 6 children treated with an antimicrobial agent within 3 days after diarrhea onset, 5 received an agent containing sulfamethoxazole, and all cases of HUS developed in children who received sulfamethoxazole. Receipt of either antimotility or antidiarrheal agents was not associated with HUS. The type of Shiga toxin (Stx 1 or 2 or both) produced by the \textit{E. coli} O157:H7 isolates was not associated with the risk of HUS.

\textbf{Discussion}.

This is the first reported nationwide study of \textit{E. coli} O157:H7 infection in the United States. Cases occurred in all geographic areas and age groups. This study was not population-based and may have oversampled adults. Nonetheless, the finding that nearly one-third of cases occurred in adults \(\geq 50\) years of age indicates that in the clinical setting of suspected infectious gastroenteritis, especially in persons with a history of bloody diarrhea, \textit{E. coli} O157:H7 infection should be considered as a cause in both children and adults in all areas of the United States.

Consumption of visibly undercooked ground beef was the only dietary factor independently associated with \textit{E. coli} O157:H7 diarrhea in multivariate analysis. Ground beef has been a common vehicle linked to \textit{E. coli} O157:H7 outbreaks.
From 1982 to 1993, of 13 reported outbreaks with an identifiable food source, 7 were linked to consumption of ground beef [2]. Among the other 6, other foods of bovine origin were implicated in 3 (roast beef 2, raw milk 1). In a study of risk factors for sporadic *E. coli* O157:H7 infections in Washington State, consumption of rare ground beef was also associated with infection [18]. The findings of the current study are further supported by two studies in Canada that linked sporadic *E. coli* O157:H7 infection with consumption of undercooked meat [24] and consumption of undercooked ground beef at picnics or special events [25]. The fact that in this study undercooked ground beef rather than ground beef per se was a risk factor for infection underscores the importance of proper cooking for prevention of *E. coli* O157:H7 infections.

We did not find any food preparation or handling practices that were linked with illness. However, a recent study of sporadic *E. coli* O157:H7 infections in New Jersey demonstrated that food preparers in households with cases were less likely to wash their hands and work surfaces after handling ground beef [26]. This suggests that public health messages encouraging thorough cooking need to be broadened to educate food preparers to wash their hands and work surfaces after contact with raw ground beef.

Although consumption of undercooked ground beef was the only variable independently associated with risk of infection with *E. coli* O157:H7 in multivariate analysis, the population-attributable risk for this behavior was only 34%. Some other cases may have been related to eating meat that was undercooked but not visibly so or may have occurred due to food exposures not asked about on our questionnaire. Recently, outbreaks of *E. coli* O157:H7 have been linked to fresh produce, specifically leaf and iceberg lettuce and unpasteurized apple cider and juice [27–29]. Prevention of *E. coli* O157:H7 infections will require increased understanding of the ecology of this organism in fresh produce and other foods.

In the current study, both drinking well water and swimming in ponds were identified as risk factors for infection in univariate analysis; among persons who did not eat undercooked ground beef, there was a trend toward an association of *E. coli* O157:H7 infection with drinking well water. Other studies have documented that swimming in or drinking unchlorinated water is a risk factor for *E. coli* O157:H7 infection [15, 16, 30–33]. The Environmental Protection Agency is considering regulations to require disinfection of all drinking water supplies [34]; until then, local unchlorinated water sources such as rural wells are potential sources for illness due to *E. coli* O157:H7 and other waterborne pathogens.

Eight percent of the patients in this study developed HUS, a proportion similar to that observed in other series [1]. In this study, no demographic variables were identified as predictors of HUS. Vomiting within 3 days of the onset of diarrhea was significantly associated with HUS, suggesting that children with this symptom should have their hematologic and renal function closely monitored. The finding that antimicrobial treatment within 3 days after diarrhea onset was associated with HUS among patients <13 years old suggests caution in the use of antimicrobial agents in these young patients. Although subinhibitory concentrations of some antimicrobial agents increase Shiga toxin production by *E. coli* O157:H7 in vitro [35], it is not known whether this phenomenon occurs in vivo or is clinically relevant. Like other retrospective studies, conclusions drawn from this study on the relationship between antimicrobial therapy for persons infected with *E. coli* O157:H7 and the subsequent development of HUS are limited by the lack of standardization of the type, timing, and duration of antimicrobial treatment, as well as the possibility that administration of antimicrobial agents could be a marker for a more severe illness [2]. Large prospective treatment trials are needed to address this question.

This study had certain limitations. First, our control group was selected from persons seeking medical care for nongastrointestinal problems, raising the possibility that dietary preferences in this group may not have been representative of well persons. However, comparison of the dietary preferences of our control group with data from population-based surveys suggests that they are comparable to those of the general population (CDC, unpublished data). Second, cases may have been more likely than controls to have heard about transmission of *E. coli* O157:H7 infection from hamburgers through contacts with health care providers or the media. We attempted to minimize this recall bias by interviewing cases as quickly as possible. In addition, this study was conducted before 1993 when a large *E. coli* O157:H7 outbreak in the western United States focused public attention on the association with hamburgers.

Because there is no proven therapy for illness due to *E. coli* O157:H7, prevention of infection is critical. Prevention of sporadic infection requires recognition and modification of risk factors such as those described here. In addition, since the infectious dose for this pathogen is low and person-to-person spread not uncommon, it is also important to identify cases to prevent secondary transmission in the household and community. Therefore, all persons with a history of acute bloody diarrhea should have a stool specimen cultured for *E. coli* O157:H7. Moreover, reporting of cases to local public health authorities can help to detect outbreaks, lead to removal of common sources of infection, and further reduce the burden of illness due to this pathogen.

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References


