An Outbreak of Enterotoxigenic *Escherichia coli* Associated with Sushi Restaurants in Nevada, 2004

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Background. In August and November 2004, 2 clusters of diarrhea cases occurred among patrons of 2 affiliated sushi restaurants (sushi restaurant A and sushi restaurant B) in Nevada. In August 2004, a stool sample from 1 ill sushi restaurant A patron yielded enterotoxigenic *Escherichia coli* (ETEC). In December 2004, we investigated a third cluster of diarrhea cases among sushi restaurant B patrons.

Methods. We defined a case as diarrhea in a person who ate at sushi restaurant B from 3 December through 13 December 2004. Control subjects were individuals who dined with case patients but did not become ill. Duplex polymerase chain reaction was used to detect genes coding for heat-stable and heat-labile enterotoxins of ETEC.

Results. One-hundred thirty patrons of sushi restaurant B reported illness; we enrolled 36 case patients and 29 control subjects. The diarrhea-to-vomiting prevalence ratio among patients was 4.5. Illness was associated with consumption of butterfly shrimp (estimated odds ratio, 7.2; 95% confidence interval, 1.1 to infinity). The implicated food was distributed to many restaurants, but only sushi restaurant B patrons reported diarrhea. We observed poor food-handling and hand hygiene practices at sushi restaurant B. Stool samples from 6 of 7 ill patrons and 2 of 27 employees who denied illness yielded ETEC.

Conclusions. ETEC was identified as the etiologic agent of a large foodborne outbreak at a sushi restaurant in Nevada. Poor food-handling practices and infected foodhandlers likely contributed to this outbreak. Although ETEC is a well-documented cause of domestic foodborne outbreaks, few laboratories can test for it. Earlier recognition of ETEC infections may prevent subsequent outbreaks from occurring.

Enterotoxigenic *Escherichia coli* (ETEC) is increasingly recognized as a cause of foodborne outbreaks of diarrhea in the United States [1–8]. Seafood was the vehicle most commonly implicated in ETEC outbreaks reported to the Centers for Disease Control and Prevention (CDC) from 1975 through 1995 [1]. We describe an outbreak of ETEC infection associated with sushi restaurants in Reno, Nevada, in 2004.

OUTBREAK

In August and November 2004, 2 clusters of diarrhea cases were documented among 34 patrons of sushi restaurant A (SR-A) and sushi restaurant B (SR-B), both part of a family-owned business in Reno, Nevada. In each cluster, patients reported diarrhea lasting from 2 to 9 days; diarrhea was reported twice as frequently as vomiting. Stool cultures tested at the Nevada State Health Laboratory (Reno, Nevada) did not yield common bacterial, viral, and parasitic pathogens. In August 2004, a stool sample from 1 ill SR-A patron, tested at the CDC, yielded ETEC serotype O127:H2.

Between 10 December and 13 December 2004, the Washoe County District Health Department (WCHD) in Reno, Nevada, received 15 reports of diarrheal illness among SR-B patrons and suspended SR-B's operation. SR-B served 800–1000 patrons per day. On 14 December 2004, after a local television report of the outbreak, WCHD received numerous calls from persons reporting diarrhea after eating at SR-B. On 15 December, we began an investigation to determine the etiologic agent, assess the magnitude of the outbreak, identify risk factors for infection, and recommend infection-control measures.

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PATIENTS, MATERIALS, AND METHODS

Case finding. We defined a case as diarrhea (⩾3 loose stools during a 24-h period) or vomiting in a person who ate at SR-B between 3 December and 13 December 2004. Cases were identified after media coverage of the outbreak on 14 December prompted calls from ill patrons to the WCHD. We inquired about clinical features, food history, and dining group size.

Case-control study. For the case-control study, we defined a case as diarrhea (⩾3 stools in a 24-h period) in a person who ate at SR-B from 3 December through 13 December 2004, with illness onset within 1 week after eating at SR-B. Control subjects were meal companions of case patients. Control subjects were excluded if they reported having diarrhea after 25 November 2004. To maximize the power of the study, we sought to identify as many case patients and control subjects as possible.

Case patients and control subjects were interviewed by telephone using a standard questionnaire from 20 December through 28 December 2004. Hypothesis-generating interviews and case report data identified commonly consumed menu items and ingredients and aided in development of the questionnaire, which addressed 50 menu items.

Environmental investigation. We observed standard operating procedures and determined adherence to food safety recommendations at SR-B. Ingredients of each menu item were recorded. We interviewed SR-B employees regarding their work-related tasks, recent illness, and travel history. We reviewed invoices, visited 2 of SR-B's seafood distributors, and discussed dissemination of the implicated ingredients with managers.

Laboratory investigation. WCHD requested stool samples from case patients who were ill at the time of their interviews. All SR-B employees were asked to submit stool samples, which were collected from 14 December through 22 December 2004.

At the Nevada State Health Laboratory, stool samples were tested for the following pathogens: *Salmonella, Shigella, E. coli* O157:H7, *Campylobacter, Yersinia, Vibrio,* Norovirus, *Cyclospora, Cryptosporidium,* and *Giardia.* Select sweeps from MacConkey agar were sent to the CDC, where duplex PCR was used to detect genes coding for the heat-labile (LT) and heat-stable (ST) enterotoxins of ETEC [9].

Employees whose stool samples yielded ETEC were asked to submit follow-up specimens. Serotyping, antimicrobial susceptibility testing, and PFGE were performed on *E. coli* colonies producing ST and/or LT enterotoxins. PCR to detect the enteroaggregative *E. coli* (EAEC) plasmid was also performed [10].

Statistical analysis. Observed group illness rates were calculated for each SR-B dining group by dividing the number of ill patrons by the total number of patrons in the group. We used exact condition likelihoods to calculate point estimates (ORs) and 95% CIs for ORs for each categorical variable, including menu items and ingredients. We created variables to capture consumption of the ingredients of each menu item included in the questionnaire. For example, if menu item X included avocado and cucumber, all patrons who ate menu item X were considered to be exposed to avocado and cucumber. Conditional logistic regression models were developed by adding variables demonstrating significance on bivariate analysis 1 by 1 in order of marginal significance. Likelihood ratio tests were used to assess the model fit of each successive multivariable model. All exposures with significant associations, substantial case exposure, and biological plausibility were examined. Statistical analyses were performed using SAS, version 9.1 (SAS Institute).

RESULTS

Case finding. During December 2004, 130 SR-B patrons reported illness to WCHD; there were no reports of illness from SR-A patrons. Case reports were completed for 113 SR-B patrons; 3 patrons ate alone, and 110 patrons were part of 66 groups. Groups consisted of 2–14 people. The median group illness rate was 75% (range, 20%–100%).

Among the 109 case patients (84%) for whom detailed information was available, the median age was 34 years (range, 19–69 years), and 41% were female. Dates of illness onset ranged from 4 December through 14 December 2004 (figure 1). Common symptoms included diarrhea and abdominal cramps; the prevalence of diarrhea was 4.5 times the prevalence of vomiting (table 1). The median incubation period was 26 h (range, 1–167 h), and the median duration of illness was 7 days (range, <1 to 17 days). There were no hospitalizations or deaths.

Case-control study. The median age and the percentage of individuals with female sex were similar between the 36 case patients (median age, 30 years; 47% female) and the 29 control subjects (median age, 35 years; 41% female). On bivariate matched analysis, 2 menu items were significantly associated with illness: the Tuna nigiri, consumed by 16 (44%) of 36 case patients and 7 (24%) of 29 control subjects (OR, 5.5; 95% CI, 1.1–54.9), and Upside Down Shrimp nigiri, consumed by 14 (39%) of 36 case patients and 7 (24%) of 29 control subjects (OR, 8.21; 95% CI, 1.2 to infinity) (table 2). The Tuna nigiri consisted of tuna and rice, whereas the Upside Down Shrimp nigiri included mayonnaise, green onions, butterfly shrimp, small scallop, tobiko (flying fish roe), and rice. In a multivariable model that included only the Tuna nigiri and Upside Down Shrimp nigiri, the model fit was significant, but neither menu item was found to be associated with illness (table 3).

On bivariate matched analysis, several ingredients were significantly associated with illness: butterfly shrimp, small scallops, tobiko, yellowtail fish, and hot chili sauce (table 2). All of these ingredients were included in the Upside Down Shrimp nigiri, except for the yellowtail fish and hot chili sauce. We

were unable to assess whether consumption of rice was associated with illness, because it was found in almost all menu items and, therefore, was consumed by most case patients and control subjects. On multivariable analysis of ingredients, the conditional logistic regression model with the best fit included only the butterfly shrimp and small scallops, and only butterfly shrimp was significantly associated with illness (table 3).

Environmental investigation. In SR-B's kitchen, sushi was extensively handled before presentation to the patron. Different employees assumed various preparation tasks, allowing for several pairs of hands to touch any single menu item. For example, to prepare the Godzilla long roll, a cook spread a yellowtail fish and mayonnaise mix on seaweed with rice by hand, rolled the seaweed, dipped the roll in tempura batter, and deep fried the roll. After frying, the rolls were immediately cooled in the refrigerator after being tightly covered. When ordered by a patron, a cook deep fried the roll again. A sushi chef then cut the Godzilla long roll into several pieces and added teriyaki sauce, hot chili sauce, green onions, and sesame seeds before a server finally presented it to the patron.

Butterfly shrimp, in addition to small scallops, yellowtail fish, and tobiko, were delivered to the restaurant frozen and were thawed before use. SR-B's distributors supplied these ingredients to other area sushi restaurants; there were no reported problems with the delivery or dissemination of their seafood. There were no reports of diarrheal illness among patrons of Reno area sushi restaurants other than SR-A and SR-B that were supplied with butterfly shrimp or any other seafood by the same distributors in 2004.

We interviewed 29 of 30 SR-B employees who worked at SR-B during November and December 2004; the thirtieth had been fired for not being punctual to work. Two sushi chefs and 3 servers reported diarrhea with illness onset in November or December 2004, prior to the outbreak; 2 of them reported working while ill. No stool samples were collected during the period of acute illness. All employees reported feeling comfortable calling in sick and were able to trade shifts when ill. There was no official sick leave policy, nor was there paid sick leave.

All employees had resided in the United States for at least 3 months before the outbreak. None reported illness among household members, foreign travel, or visitors from another country within 1 month before the outbreak. At SR-B, employees often ate food prepared by one another. Eighteen employees reported working at SR-A at least once since starting to work at SR-B. Eight of these employees, including 1 cook,

Table 1. Clinical characteristics of ill patrons of sushi restaurant B, Washoe County, Nevada, December 2004.

Clinical characteristic	No. (%) of ill patrons (n = 109)
Diarrhea	109 (100)
Abdominal cramps	102 (94)
Nausea	68 (62)
Headache	60 (55)
Chills	58 (53)
Fever	43 (40)
Vomiting	24 (22)
Blood in stool	6 (6)
Sought medical care	27 (25)
Received antibiotics	14 (13)
Received intravenous fluids	2 (2)

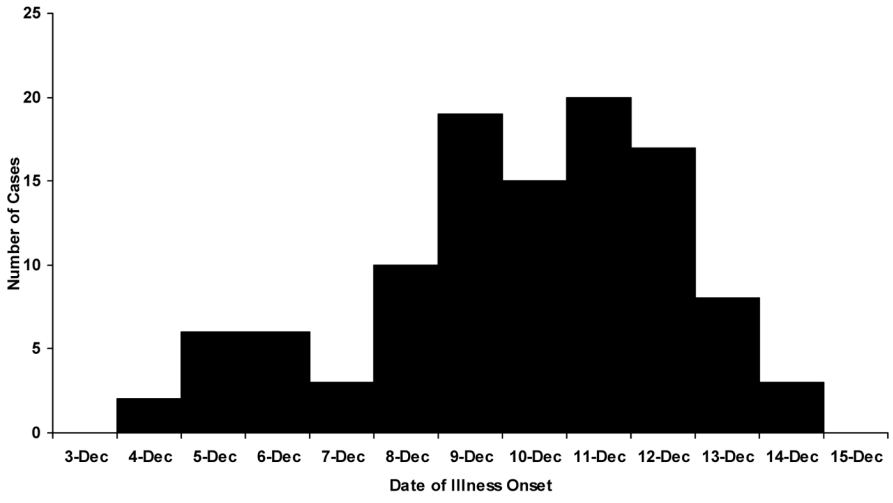

Figure 1. Number of cases of gastrointestinal illness among patrons who ate at sushi restaurant B, by date of illness onset. Washoe County, Nevada, December 2004 (n = 109).

Table 2. Bivariate analysis of selected exposures among case patients and control subjects who ate at sushi restaurant B, Washoe County, Nevada, December 2004.

Exposure	No. (%) of case patients (n = 36)	No. (%) of control subjects (n = 29)	Estimated OR (95% CI)[a]
Menu item			
Tuna nigiri[b]	16 (44)	7 (24)	5.5 (1.1–54.9)
Upside Down Shrimp nigiri[c]	14 (39)	7 (24)	8.21[d] (1.2 to infinity)
Ingredient			
Mayonnaise	36 (100)	26 (90)	7.0[d] (0.7 to infinity)
Green onions	33 (92)	22 (76)	8.4 (0.99–394.2)
Tobiko (flying fish roe)	30 (83)	17 (59)	11.7 (1.6–513)
Hot chili sauce	32 (89)	21 (72)	8.0 (1.0–374.5)
Tuna	30 (83)	20 (69)	8.3 (0.95–394.1)
Small scallops	28 (78)	13 (45)	8.3 (1.6–85.4)
Yellowtail fish	25 (69)	14 (48)	5.9 (1.2–58.8)
Butterfly shrimp	21 (58)	9 (31)	13.2[d] (2.1 to infinity)

NOTE. Overall, the rate of missing variables was very low.

[a] Point estimates are maximum likelihood unless otherwise indicated. The likelihood is the exact conditional likelihood and thus CI's are exact.

[b] Tuna nigiri ingredients were tuna and rice.

[c] Upside Down Shrimp nigiri ingredients were mayonnaise, green onions, butterfly shrimp, small scallops, tobiko, and rice.

[d] OR is a median unbiased estimate.

1 sushi chef, and 6 servers, reported working at both restaurants for at least 1 day in November or December 2004.

After the clusters of diarrhea cases in August and November 2004, frequent sanitary inspections were conducted by WCHD, and specific recommendations were made to improve food handling practices at both restaurants. In August 2004, SR-A complied with temperature regulation recommendations. The owners did not comply with a recommendation to close both restaurants to do a thorough cleaning. In November 2004, improper cooling techniques were found at SR-B, and sushi chefs were found to be using personal knives that were not properly sanitized. At the time of the December 2004 outbreak, aberrancies were still found in temperature regulation and in sanitization of sushi chef knives. However, there were no unique practices directly correlated with the preparation of items found to be associated with illness in the outbreak, including butterfly shrimp.

In December 2004, inconsistent use of gloves and inconsistent hand washing were observed at SR-B, despite adequate provision of sinks, gloves, and hand hygiene products. In August 2004, after the first cluster of diarrhea cases, WCHD emphasized strict hand washing for all employees and implemented a mandatory glove policy for cooks at both restaurants. In November 2004, the glove policy was expanded to include sushi chefs. In addition, employees were reminded that glove use was not a substitute for hand washing and that both aspects of hand hygiene were required. There was also improved signage, in Spanish and English, to remind workers to wash their hands in bathrooms and in the kitchen. Despite these policies, we found SR-B employees using the same gloves for many hours, without replacement when changing tasks or location. In addition, inconsistent hand washing between glove changes was observed.

Laboratory investigation. At the Nevada State Health Laboratory, stool samples from 14 patients and 29 employees were tested; all samples had test results that were negative for common bacterial, viral, and parasitic enteric pathogens. Sweeps of MacConkey agar were selected from the stool cultures of 7 patients whose stool samples were collected within 5 days after symptom onset and who had not been treated with antibiotics. Because no employees reported symptoms at the time that samples were collected, sweeps from MacConkey agar were collected from the cultures of 27 employees that showed growth of *E. coli;* 2 employees had stool cultures that showed no growth of *E. coli*, so sweeps from MacConkey agar could not be obtained for these 2 patients.

LT or ST enterotoxins of ETEC were detected by duplex PCR from sweeps of MacConkey agar for 6 patients and 2 employees. Multiple ETEC serotypes were detected; the most common was ETEC serotype O6:H16 (LT+ ST+), which was isolated from 3 patients and employee X (figure 2). PFGE patterns for all isolates of the same serotype were indistinguishable by 2 restriction enzymes, *Xba*I and *Bln*I. Thirty percent of isolates demonstrated antimicrobial resistance; all of these isolates were resistant to 4 agents, including ampicillin, chloramphenicol, nalidixic acid,

Table 3. Multivariable analysis of selected exposures among matched case patients and control subjects who ate at sushi restaurant B, Washoe County, Nevada, December 2004.

Model, exposure	No. (%) of case patients (n = 36)	No. (%) of control subjects (n = 29)	Estimated OR (95% CI)[a]
Model 1			
Tuna nigiri	16 (44)	7 (24)	3.8 (0.7–39.4)
Upside Down Shrimp nigiri	14 (39)	7 (24)	5.7 (0.8 to infinity)
Model 2			
Butterfly shrimp	21 (58)	9 (31)	7.2[b] (1.1 to infinity)
Small scallops	28 (78)	13 (45)	5.2 (0.8–60.7)
Model 3			
Butterfly shrimp	21 (58)	9 (31)	4.2[b] (0.5 to infinity)
Small scallops	28 (78)	13 (45)	3.4 (0.4–41.4)
Tobiko (flying fish roe)	30 (83)	17 (59)	2.3 (0.2–124.2)
Yellowtail fish	25 (69)	14 (48)	2.7 (0.4–32.8)

[a] Point estimates are maximum likelihood, unless otherwise indicated. The likelihood is the exact conditional likelihood, and therefore 95% CIs are exact.
[b] OR is a median unbiased estimate.

streptomycin, sulfisoxazole, and trimethoprim-sulfamethoxazole (figure 2). There was no resistance to flouroquinolones.

Employee X's second stool sample, obtained 1 month after collection of the initial stool sample, revealed ETEC O6:H16. After treatment with ciprofloxacin, his third stool specimen was ETEC negative. Employee Y's second stool sample, obtained 2 months after collection of the initial stool sample and initiation of ciprofloxacin treatment, was ETEC negative.

EAEC was detected by PCR in stool samples obtained from 4 patients, all of whom had test results positive for ETEC, and in stool samples obtained from 6 asymptomatic employees, all of whom had test results that were negative for ETEC. EAEC was not detected in stool samples obtained from any of the ETEC-positive employees.

DISCUSSION

We investigated the third cluster of diarrhea cases among patrons of 2 family-owned sushi restaurants in Reno, Nevada, in 2004; ETEC was identified in 2 of these clusters. Employee crossover between restaurants and poor food-handling practices likely contributed to repeated ETEC outbreaks at these restaurants. The December 2004 outbreak was limited to SR-B patrons, and there were no reports of illness from patrons of other area sushi restaurants, including SR-A, during the same period.

Although ETEC is a well-documented cause of foodborne outbreaks in the United States, few laboratories can test for ETEC, and it is often unrecognized as the etiologic agent in clusters of diarrhea cases [1, 5]. Symptom profiles in these sushi restaurant–associated clusters were consistent with ETEC. In the absence of other enteric pathogens in stool samples from ill persons, the longer duration of illness, compared with that of viral gastroenteritis, and the high diarrhea-to-vomiting ratio are key to suspecting ETEC [1]. The CDC serves as a reference laboratory and can lend expertise to state public health laboratories if testing for ETEC is unavailable.

In this outbreak, multiple ETEC serotypes were discovered, which is a common finding in ETEC outbreaks [1], but serotype O6:H16 (LT+ ST+) was predominant. The antimicrobial resistance pattern detected was consistent with recently reported trends [1, 2, 11]. Although EAEC was detected, there were no symptomatic patrons or employees in whom EAEC was detected and in whom ETEC was not detected. Therefore, we concluded that EAEC was not the likely etiologic agent of diarrhea in this outbreak.

Because no specific menu item was significantly associated with illness on multivariable analysis, we also performed an ingredient analysis. Of the 5 ingredients that were implicated on bivariate analysis, only butterfly shrimp, which was implicated in 61% of cases, remained significantly associated with illness on multivariable analysis. Butterfly shrimp, like other seafood served at SR-B, was imported and could have become contaminated with ETEC during processing. However, distributors supplied butterfly shrimp to many area restaurants, and contamination at the processing level would have led to a more widespread outbreak. During December 2004, there were no reports of illness among patrons of other Reno-area sushi restaurants, including SR-A, pointing to sources of infection at SR-B, rather than a contaminated ingredient supplied to the restaurant. We were unable to ascertain any unique practices involved in handling the butterfly shrimp that could have resulted in its contamination.

Employee crossover between these 2 family-owned restaurants could have contributed to the clusters of infection among

Identification	ETEC serotype/ toxin type	Antimicrobial resistance	Pulsed-field gel electrophoresis pattern
Patient A	Ound:H7 / LT+	Sensitive	
Patient B	O128:H12 / ST+	Ap, Na, Su, TmS	
Patient C	O128:H12 / ST+	Ap, Na, Su, TmS	
Patient D	O79:H10 / LT+ (2 colonies) O6:H16 / LT+ ST+ (3 colonies)	Ap, Ch, St, Su Sensitive	O79:H10 O6:H16
Patient E	O6:H16 / LT+ ST+	Sensitive	
Patient F	O6:H16 / LT+ ST+	Sensitive	
Employee X	O6:H16 / LT+ST+	Sensitive	
Employee Y	O152:H10 / ST+	Sensitive	

Figure 2. Centers for Disease Control and Prevention laboratory analysis of isolates obtained from patients and sushi restaurant B employees with enterotoxigenic *Escherichia coli*–positive stool samples, Washoe County, Nevada, December 2004. PFGE patterns of *Xba*I restriction enzyme are shown. Ap, ampicillin; Ch, chloramphenicol; LT, heat-labile toxin; Na, nalidixic acid; St, streptomycin; ST, heat-stable toxin; Su, sulfisoxazole; TmS, trimethoprim-sulfamethoxazole.

patrons at both restaurants in August 2004 and among patrons of SR-B in December 2004. Strong food-handling guidelines may have been successfully implemented at SR-A in August 2004; however, these were not implemented at SR-B, possibly leading to the third cluster of infection, which occurred only among SR-B patrons. Five SR-B employees reported having diarrheal episodes in November or December 2004 prior to the onset of illness among SR-B patrons involved in the outbreak. Although SR-B employees reported feeling comfortable calling the employer to report illness, there was little motivation, without an official sick leave policy or paid sick leave, to abstain from working while ill. All 5 ill employees returned to work immediately after symptoms resolved and, if they were infected with ETEC, they could have shed bacteria for another 3–6 days [7, 8, 12–14]. One of these employees had test results that were positive for ETEC serotype O6:H16, which was indistinguishable by PFGE from the O6:H16 isolates obtained from 3 patients. Although the SR-B cooks reported wearing gloves while preparing food, we observed that employees were not washing hands between glove changes. Using gloves has often failed to reduce bacterial contamination of foods, because food workers often wear the same pair for a variety of tasks over a prolonged period of time without adequate hand washing between glove changes [15, 16]. It is plausible that an ill employees' hands became contaminated with ETEC because of contact with his or her stool, and that his or her hands or gloves subsequently contaminated the butterfly shrimp, leading to this outbreak. Cross-contamination attributable to poor hand hygiene likely contributed to multiple vehicles of ETEC transmission in this outbreak.

There were limitations to this investigation. Through case finding, we identified 130 case patients, 109 of whom provided detailed information. However, because of the high group illness rate, only 36 case patients and 29 control subjects could be enrolled in the matched case-control study, which reduced the power of the study. We were unable to test any food samples for enteric pathogens, because all foods had been discarded by the start of the formal investigation. For employees who reported diarrheal illness, stool samples were obtained >2 weeks after their reported episodes, lowering the likelihood of recovering enteric pathogens, including ETEC.

In summary, ETEC was the etiologic agent of a large foodborne outbreak among patrons of a sushi restaurant in Reno, Nevada, in December 2004. We identified butterfly shrimp as the food vehicle for ETEC transmission during this outbreak. Poor food-handling practices and infected foodhandlers likely contributed to ongoing transmission of the pathogen at SR-B. Restaurant owners should encourage employees not to work while ill and should provide incentives for compliance. In addition, appropriate hand hygiene in the workplace must be promoted and facilitated. Although ETEC was identified in only 1 of 2 prior documented clusters of diarrhea cases associated with SR-A and SR-B, the clinical profile of all 3 clusters of infection is compatible with ETEC. Recognition of the typical clinical profile of ETEC infection can lead to earlier identifi-

cation of ETEC through state and federal public health laboratories.

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