

## Laboratory and Epidemiology Communications

# A Case of Hemolytic-Uremic Syndrome Associated with Shiga Toxin 2-Producing *Escherichia coli* O121 Infection Caused by Drinking Water Contaminated with Bovine Feces

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Communicated by Kazuo Kato

(Accepted November 11, 2002)

Shiga toxin (*stx*)-producing *Escherichia coli* (STEC) is an important cause of hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) in humans (1). STEC O157:H7 is the serotype most commonly isolated from HUS patients, but non-O157 STEC have also been implicated in HC and HUS cases (2,3). Cattle are known as carriers of both O157 and non-O157 STEC (4,5). STEC infection is primarily a food-borne disease. Foods, such as unpasteurized milk, undercooked hamburgers, salad, and white radish sprouts, have been identified as vehicles in STEC infection outbreaks (6). However, vehicles have rarely been identified in sporadic cases of either O157 or non-O157 STEC infections. We report here an HUS case in Akita Prefecture that was caused by an *stx 2* gene (*stx2*)-positive STEC O121. The case involved family members and the vehicle was traced to contaminated drinking well water.

Mackonkey agar supplemented with Cefixime Tellurite supplement (Oxoid, Basingstoke, England) (CT-Mac) was used to select STEC O121, which formed distinctive clear and slightly brown-colored colonies on the agar. CT-Mac and EC broth were inoculated with stool specimens and incubated overnight at 37°C. Three liters of drinking water samples were filtered through 0.45  $\mu$ m pore-size membrane filters, which were then placed in trypticase soy broth and incubated overnight at 35°C. Environmental swabs were cultured and incubated in the same manner as the membrane filters. Manure and bovine feces were cultured and incubated in the same manner as the stool specimens. Each culture broth was analyzed by PCR using VTcom primers in order to screen for *stx*-positive strains (7). The *stx* and *E. coli* attaching and effacing (*eaeA*) gene were typed and detected, respectively, by PCR as described previously (7). Pulsed-field gel electrophoresis (PFGE) analysis of the STEC isolates was performed using *Xba*I digests of the chromosomal DNAs (7).

A 2-year-old girl developed watery diarrhea on October 3, 2000, which progressed to bloody diarrhea on October 5. She was hospitalized on October 6, 2000, and developed HUS on October 7. The hospital laboratory sent us an *E. coli* isolate from the patient's stool for *stx* evaluation on October 7. The strain was identified as *stx2*-positive STEC O121. A hospital doctor notified the local public health center of the STEC O121 HUS case on October 7. Stool specimens collected from the family members by the officials were sent to us on October

7 for STEC examination.

As shown in Table 1, the examination revealed that the patient's father and brother were infected with *stx2*-positive STEC O121, while the patient's mother and aunt were infected with *stx1*-positive STEC O91, indicating that the case was an intra-family infection involving two STEC serotypes. None of the individuals infected with STEC O121 or O91 showed clinical symptoms. All of the STEC O121 isolates were *eaeA* positive, while neither of the two STEC O91 isolates were positive for the gene.

The local public health center officials' epidemiological investigation revealed that the family utilized un-chlorinated well water that was pumped up at a well and sent through 80 m long piping to their house. Seven cattle were raised in a cattle shed near the well. The well was impaired so that surface water could flow into the well. The local public health center officials collected water samples, environmental samples, and bovine feces and sent them to us for STEC examination. Table 2 summarizes the results of the STEC evaluation of these samples. The drinking water samples collected on October 7 and the water of the well and the tap water in the patient's house collected on October 11 were positive for *stx2*-harboring STEC O121. The tap water sample collected on October 10 was STEC negative, indicating that the water was intermittently contaminated with STEC O121. Cows No. 5 and No. 7 were, respectively, positive for *stx2*-positive STEC O121 and *stx1*-positive STEC O111. No other cattle were positive for STEC, including STEC O91.

The *stx2*-positive STEC O121 strains isolated from the patient, the family members, water samples, and bovine feces were subjected to *Xba*I PFGE pattern analysis. The STEC O121 strains had identical *Xba*I PFGE patterns, indicating that they were of a common origin. From these

Table 1. Results of evaluation for STEC in stool specimens obtained from patient's family on October 7, 2000

Relationship to patient	Results
Father	STEC O121 <i>stx2</i> + positive
Mother	STEC O91 <i>stx1</i> + positive
Brother	STEC O121 <i>stx2</i> + positive
Grandmother	STEC negative
Great-grandmother	STEC negative
Aunt	STEC O91 <i>stx1</i> + positive
Cousin	STEC negative

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Table 2. Results of evaluation for STEC in drinking water, environmental samples, and bovine feces

Samples	Collection date	Results
Drinking water	Oct. 7, 2000	STEC O121 <i>stx2+</i> positive
Drinking water <sup>1)</sup>	Oct. 10, 2000	STEC Negative
Drinking water <sup>2)</sup>	Oct. 11, 2000	STEC O121 <i>stx2+</i> positive
Drinking water <sup>1)</sup>	Oct. 11, 2000	STEC O121 <i>stx2+</i> positive
Drinking water <sup>1)</sup>	Oct. 31, 2000	STEC Negative
Drinking water <sup>2)</sup>	Oct. 31, 2000	STEC Negative
Manure No.1	Oct. 7, 2000	STEC Negative
Manure No.2	Oct. 31, 2000	STEC Negative
Manure No.3	Oct. 31, 2000	STEC Negative
Environmental swabs <sup>3)</sup>	Oct. 7, 2000	STEC Negative
Tap water of cow house	Oct. 31, 2000	STEC Negative
Ditch water No.1 <sup>4)</sup>	Oct. 10, 2000	STEC Negative
Ditch water No.2 <sup>4)</sup>	Oct. 31, 2000	STEC Negative
Ditch water No.3 <sup>4)</sup>	Oct. 31, 2000	STEC Negative
Bovine No.1 feces	Oct. 31, 2000	STEC Negative
Bovine No.2 feces	Oct. 31, 2000	STEC Negative
Bovine No.3 feces	Oct. 31, 2000	STEC Negative
Bovine No.4 feces	Oct. 31, 2000	STEC Negative
Bovine No.5 feces	Oct. 31, 2000	STEC O121 <i>stx2+</i> , STEC O111 <i>stx1+</i> positive
Bovine No.6 feces	Oct. 31, 2000	STEC Negative
Bovine No.7 feces	Oct. 31, 2000	STEC O121 <i>stx2+</i> , STEC O111 <i>stx1+</i> positive

<sup>1)</sup> Collected at the well.

<sup>2)</sup> Tap water collected at the patient's house.

<sup>3)</sup> Obtained in the patient's house: 12 swab samples were collected and subjected to STEC examination.

<sup>4)</sup> Collected downstream of the cow house drainage influx point.

and the epidemiological investigation results, we concluded that the well water contaminated with *stx2*-positive STEC O121 was the vehicle in this infectious case. The well water was possibly contaminated by the influx of surface water containing the contaminated bovine feces.

O121 is a rare STEC serotype associated with human diseases in Japan, but a few infectious cases have been identified yearly in this prefecture since 1997 (7). This is the first HUS case associated with STEC O121 and none of the other non-O157 STEC strains has been associated with the development of HUS in this prefecture. HUS outbreak associated with STEC O121 infection was also reported in Connecticut, U.S. in 1999 (8) and a few other cases of HUS associated with STEC O121 have also been reported (9,10). O121 should be considered an important STEC serotype that causes HUS, a serious public health problem.

We previously reported a familial infectious case associated with *stx1*-positive STEC O103:H2, and a cow was identified as an infectious source as in this case (11). The significance of cattle as infectious sources of human STEC infections should be further investigated.

This article appeared in the Infectious Agents Surveillance Report, vol. 22, no. 6, p. 141-142, 2001 in Japanese.

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