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An Outbreak of Shigatoxin-Producing *Escherichia coli* O157:H7 in a Nursery School in Mie Prefecture

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A major outbreak of Shigatoxin-producing *Escherichia coli* (STEC) O157:H7 infection occurred throughout Japan in 1996 (1). Since then, the number of individuals infected has declined to the 1,000 individuals seen annually (2). STEC infection often occurs in the form of food poisoning caused by the consumption of bacteria-contaminated food (3,4), but this infection is also characterized by the occurrence of secondary infection in which a small number of bacteria allow establishment of the infection (3,4). In late July 2004, a 1-year-old nursery school pupil in Inabe, Mie Prefecture, with a mucoid, bloody diarrhea stool demonstrated STEC O157:H7 infection. Thereafter, other pupils in this pupil's class were found to be infected. Additional pupils presenting similar findings continued to appear in the nursery school, and suspicion arose of institutional infection spreading to families of the pupils. We report a summary of these cases.

An interview investigation was conducted at the school, and the pupils, employees, and families of the initial class underwent health examinations and stool examinations. One platinum loop of stool was streaked on a CT-SMAC agar plate medium and inoculated with mEC broth. The material was then isolated and cultured on a CT-SMAC agar plate medium (5). Approximately 10 of the gray, semitransparent sorbitol non-decomposing colonies that proliferated on the medium were checked for the presence oh the Shigatoxin producing gene (*stx*) by PCR (6). Positive colonies underwent biochemical investigation by the IMViC system and were then serotyped by agglutination reaction with O and H anti-serum (Denka Seiken, Ltd. Co., Tokyo, Japan) (7). Pink, sorbitol-decomposing colonies were also collected in a similar fashion to isolate STEC of serotypes other than O157, and the presence of *stx* was investigated by PCR (1,4). Pure, cultured bacteria were then processed by a *Bln*I restriction enzyme for the analysis by pulsed-field gel electrophoresis (PFGE) with Bio-Rad CHEF-DRII (Bio-Rad Laboratories, Hercules, Calif., USA) (8).

The initial case, No. 1, presented with fever on July 29, 2004, and passed bloody stool beginning on August 1. *stx*2-carrying STEC O157:H7 was detected in the patient's stool on August 3. Class A, the same class in the nursery school that the patient had attended, included numerous pupils with diarrhea symptoms. It was also discovered that a hospitalized nursery school pupil presenting diarrhea, bloody stool, and fever since July 27 and negative for STEC O157:H7 on stool investigation had hemolytic uremic syndrome (HUS). An investigation was then carried out on August 8 by visits to every house of the nursery school's employees and the Class A pupils and their families. Isolated instances of pupils with diarrhea beginning in early July were discovered, and at the time of the survey, more than half of the pupils had diarrhea. In contrast, 1 - 2 pupils in classes of age 2, 3, 4, and 5 years had diarrhea. Different pools were used by each class, and to avoid skin irritation among the infants, a chlorine agent was added to all pools except the baby pool used by Class A.

Table 1 presents the results for each group investigated. *stx*2-carrying STEC O157:H7 was isolated from 13/23 (56.6%) pupils 0-1 years old in Class A of the initial patient. At the same time, investigation among 4 family members of the initial patient found no symptomatic individuals, and stool results were negative for all individuals. On August 8-13, stool investigations were completed for all nursery school pupils, employees, and families of Class A pupils, and a total of 22 new *stx*2-carrying STEC O157:H7 infections were discovered, 15 in school pupils, 1 in an employee, and 6 among

Table 1. Isolation of Shigatoxin-producing *Escherichia coli* O157:H7*

Group by age (year)	No. of examination	No. of positive	Positive percentage
0 - 1 year-old pupil (Class A)	23	13	56.5
2-year-old pupil	23	0	0
3-year-old pupil	27	0	0
4-year-old pupil	34	1	2.9
5-year-old pupil	33	2	6.1
Class A pupil's family	70	6	8.6
Family members except Class A pupil	39	0	0
Staff	30	1	3.3
Total of stool examination	279	23	8.2
Preservation food	22	0	0
Wiping of the food preparation room	13	0	0

*: All isolates were *stx*2-carrying.

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family members. In PFGE performed on bacteria isolated from 23 total individuals, all of the strains demonstrated an indistinguishable pattern. As shown in Table 2, bacteria were isolated from 6 family members, and all of these individuals were family of Class A pupils. With the exception of No. 20, patients among family members and nursery care providers were limited to children age 7 years and under. STEC O157:H7 was not detected either from stored food of meals provided between July 21 and July 31 (22 samples) or from wipes of the food and milk preparation rooms in the school (13 samples).

In this outbreak, epidemiological investigation and laboratory tests for stored food and wipe samples did not indicate food preparation as the cause, and the source of infection in the initial case could not be determined. Cases of diarrhea among the school pupils began in early July, but these were not investigated, and the source for the infection by STEC O157:H7 could not be determined. However, in light of the long latency period involved, and the fact that more than 1 month is required from manifestation in the initial patient until negativity is reached in all cases, there is a strong possibility that some pupils were infected in July, and STEC O157:H7 was excreted thereafter for a long duration. When STEC O157:H7 infects infants, the rate of occurrence is high, and occasional causation of HUS is not uncommon (9). However, progress is sometimes asymptomatic, despite infection. STEC O157 survives for very long durations even in water and other environments, and even a bacterial quantity of approximately 2 logCFU is reportedly sufficient to establish infection (3). If infants are diapered, there may be a delay until diarrhea is discovered, and the child may be sent to school despite some amount of diarrhea, which also suggests the possibility that infection occurred during a diaper change, or laterally by way of a pool (fecal-oral). Contamination of the baby pool by bacteria excreted by an infected individual and infection of other, healthy individuals using the pool is also conceivable. Passage of infection to families may have occurred through underwear soiled with feces or through contact within the family. STEC infection is often discovered in the form of food poisoning in meat or other foods contaminated with the bacteria, or in undercooked food, but there are also reported cases of institutional lateral infection, such as we have encountered, in old age facilities (10), child mental health centers (8), child daycare centers (11), and nursery schools (12). When infected individuals are discovered in such settings, efforts to prevent primary infection through routine management of food preparation are of course important, as is prevention of secondary infection through the spread of or contact with feces of an infected individual.

Table 2. The investigated data of Shigatoxin-producing *Escherichia coli* O157:H7 infected patients and carriers

No.	Classification	Attribute	Age	Sex	Patient/ carrier	Determined date*
1	pupil	class A	1	M	patient	7/8/04
2	pupil	class A	1	F	patient	9/8/04
3	pupil	class A	1	F	patient	9/8/04
4	pupil	class A	1	M	patient	9/8/04
5	pupil	class A	0	F	patient	9/8/04
6	pupil	class A	1	M	patient	9/8/04
7	pupil	No.6's elder sister	5	F	patient	10/8/04
8	pupil	class A	1	F	patient	10/8/04
9	pupil	No.8's elder brother	4	M	patient	10/8/04
10	pupil	class A	1	M	patient	10/8/04
11	pupil	class A	1	M	patient	10/8/04
12	pupil	class A	1	F	patient	10/8/04
13	family	No.8's grandmother	59	F	carrier	10/8/04
14	pupil	class A	1	F	patient	11/8/04
15	pupil	class A	1	M	patient	11/8/04
16	pupil	class A	1	M	patient	11/8/04
17	family	No.12's mother	29	F	carrier	11/8/04
18	pupil	No.16's elder brother	5	M	patient	12/8/04
19	family	No.16's elder brother	7	M	patient	12/8/04
20	family	No.16's mother	31	F	patient	12/8/04
21	family	class A pupil's father	24	M	carrier	12/8/04
22	family	class A pupil's father	37	M	carrier	12/8/04
23	staff	nurse	28	F	carrier	12/8/04

*: Day/Month/Year.

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