

## Laboratory and Epidemiology Communications

### Outbreak of Enterohemorrhagic *Escherichia coli* O121 among School Children Exposed to Cattle in a Ranch for Public Education on Dairy Farming

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In 2004 June, there was an outbreak of enterohemorrhagic *Escherichia coli* (EHEC) O121 infections among school children exposed to infected cattle in a ranch for public education on dairy farming in Chiba Prefecture, Japan.

The first two patients had diarrhea and abdominal pain on June 13 and 14. VT2 producing EHEC was isolated but the O serogroup was untypable (OUT). A total of 63 school children in the 6th grade of the U primary school developed

symptoms. They were among 110 persons (101 children and 9 teachers) that attended a summer school in K City in Chiba Prefecture from June 9 to 11. During the same period of time, there was a report of EHEC infection (OUT) from a kindergarten. The common exposure point for all of the affected school and kindergarten children was the R ranch for public education on dairy farming in Chiba Prefecture. The investigation of the public authority of Chiba Prefecture revealed that the stool samples of all six cattle kept at the ranch and soil specimens from the cattle pen contained EHEC OUT, and that some of both specimens also contained EHEC O157. An investigation by the Akita Prefectural Institute

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of Public Health identified that the EHEC OUT previously isolated from the school children was EHEC O121.

We inoculated CHROMagar O157, CT-SMAC and DHL plates with specimens directly and also after amplification in TSB broth. The isolated EHEC O121 developed white colonies on DHL plates, and they appeared to be a mixture of lactose-fermenting and non-fermenting bacteria. A VT-production test using polymyxin B was performed as described previously (1). EHEC O121:H19 (VT2) was isolated from 15 patients and 2 asymptomatic individuals, and EHEC O157:H7 (VT1 and 2) was isolated from 2 affected children. Among them, one patient had both EHECs.

Pulsed-field gel electrophoresis (PFGE) (2) of *Xba*I-digested chromosomal DNA revealed that all the EHEC O121 isolates had the same PFGE pattern, except one patient-derived isolate that differed from the others by three bands, and two soil-derived isolates that differed from the others by two bands (Fig. 1). The EHEC O157 isolates had similar PFGE patterns; one patient isolate and the bovine fecal isolate (lanes 21 and 23, Fig. 1) had identical patterns. The other patient-derived isolate and the soil-derived isolate (lanes 22 and 24, Fig. 1) differed from the former two by one (not identical) band. The above results are summarized in Table 1. Antimicrobial susceptibility testing done by the KB method (3) revealed that all the isolates were sensitive to 12 antibiotics, i.e., ampicillin, cefotaxime, kanamycin, gentamicin, streptomycin, tetracycline, chloramphenicol, norfloxacin, nalidixic acid, fosfomycin, trimethoprim, and trimethoprim-sulfamethoxazole.

Investigation by the public authority of Chiba Prefecture revealed the following. First, the school children made butter

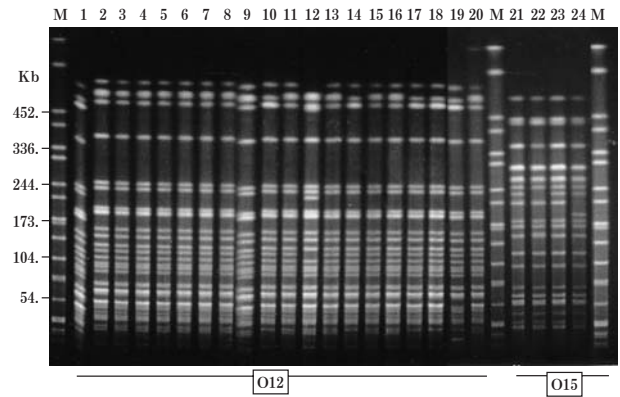


Fig. 1. PFGE patterns of *Xba*I restriction fragments of chromosomal DNA of EHEC O121:H19 and O157:H7 isolates. Lanes 1-17: specimens derived from children in U primary school (O121), Lane 18: cattle stool specimens (O121), Lane 19: soil specimen from the cattle pen (O121), Lane 20: specimen derived from patient in K city (O121), Lanes 21-22: specimens derived from children in U primary school (O157), Lane 23: cattle stool specimens (O157), Lane 24: soil specimen from the cattle pen (O157), M: *Salmonella* Braenderup H9812.

and soybean powder candies after lunch at the ranch. Second, the children were permitted to touch the cows through a fence. Third, the ranch had only a limited number of hand-washing facilities, which were insufficient for all the children. Fourth, cattle dung was scattered in the surroundings of the cattle pens; the sanitary measures were insufficient. From these circumstances and also from the isolation of EHECs from the environment, it was concluded that the outbreak was

Table 1. EHEC strains isolated from patients and bovine sources

Strain No.	Isolation date	Age/Sex	Gastrointestinal symptoms			Characteristics of isolates		
			Diarrhea	Bloody diarrhea	Abdominal pain	Sero type	toxin	PFGE type
1	2004/6/19	11/F	○	○	○	O121:H19	VT2	A
2	2004/6/19	10/M	○		○	O121:H19	VT2	A
3	2004/6/19	11/F	○	○	○	O121:H19	VT2	A
4 <sup>1)</sup>	2004/6/21	11/F			○	O121:H19	VT2	A
5	2004/6/22	11/F	○		○	O121:H19	VT2	A
6	2004/6/22	11/F	○		○	O121:H19	VT2	A
7	2004/6/22	11/M			○	O121:H19	VT2	A
8	2004/6/22	11/M	○		○	O121:H19	VT2	A
9	2004/6/22	11/F	○		○	O121:H19	VT2	A
10	2004/6/22	11/F	○			O121:H19	VT2	A
11	2004/6/22	11/F	○			O121:H19	VT2	A
12	2004/6/22	11/M			○	O121:H19	VT2	A'
13	2004/6/23	11/F	○		○	O121:H19	VT2	A
14	2004/6/24	11/M	○		○	O121:H19	VT2	A
15	2004/6/25	11/F	○		○	O121:H19	VT2	A
16	2004/6/25	11/F		None		O121:H19	VT2	A
17	2004/6/25	11/M		None		O121:H19	VT2	A
18 <sup>2)</sup>	2004/7/7					O121:H19	VT2	A
19 <sup>3)</sup>	2004/7/7					O121:H19	VT2	A''
20 <sup>4)</sup>	2004/6/17	3/F				O121:H19	VT2	A
21 <sup>1)</sup>	2004/6/21	11/F			○	O157:H7	VT&2	B
22	2004/6/22	11/F	○		○	O157:H7	VT&2	B'
23 <sup>2)</sup>	2004/7/7					O157:H7	VT&2	B
24 <sup>3)</sup>	2004/7/7					O157:H7	VT&2	B''

Strain Nos. 1-17, 21-22: children in U primary school.

<sup>1)</sup>: Same patient. <sup>2)</sup>: Bovine feces. <sup>3)</sup>: Soils around cowsheds. <sup>4)</sup>: Resident within K city.

caused through contamination of the school children's hands with EHEC O121 derived from the infected cattle.

Outbreaks caused by EHEC O121, though rare, have been reported in Akita and Saga Prefectures (4-5). In situations in which humans and cows come into close contact, EHEC O121 infection can occur, and special care, such as environmental sanitation and hand washing, should be taken. The present outbreak was caused by EHEC of a rare serotype. The prompt response of the medical facilities and quick response of the responsible authorities in Chiba City and Chiba Prefecture facilitated identification of the source of the outbreak.

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