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Short Communication

Phage Typing and DNA-Based Comparison of Strains of Enterohemorrhagic *Escherichia coli* O157 from Apparently Sporadic Infections in Osaka City, Japan, 1996

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SUMMARY: A marked increase in sporadic cases of enteritis due to enterohemorrhagic *Escherichia coli* serogroup O157 occurred in Osaka City, Japan, during 1996. To elucidate why the number of cases had increased, the isolates were classified using phage typing, random amplified polymorphic DNA analysis, and pulsed-field gel electrophoresis (PFGE). Fifty-seven percent of the isolates (105/184) belonged to the same phage type (PT-32) and gave the same PFGE pattern; the clone had been isolated during a 3-week period, with a peak on July 15. It was concluded that the majority of the cases identified in July 1996 formed an outbreak, although epidemiological links to a possible common source were not established. The possibility that this outbreak was part of a huge regional outbreak including children at primary schools in Sakai City was discussed.

The first outbreak due to enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 in Japan occurred in September 1990 (1). This was followed by a second outbreak in a day-care center in Osaka City in 1991; 161 individuals were identified as cases or healthy carriers. However, between 1992 and 1995 only four sporadic cases were reported in Osaka City. From May 1996, outbreaks due to EHEC O157 were reported throughout Japan. Then a massive outbreak occurred in the primary schools in Sakai City, Osaka Prefecture (2). Sakai City borders Osaka City, and the number of sporadic cases in Osaka City also increased suddenly in July 1996.

The aim of this study was to investigate in detail the isolates from the apparently sporadic cases of EHEC O157 infection in Osaka City, using phenotypic and DNA-based methods, and to determine whether any of the cases constituted part of a bigger picture. The advent of molecular typing strategies has benefited epidemiological investigations, by way, for example, of the use of restriction fragment length polymorphisms (3), pulsed-field gel electrophoresis (PFGE)(4,5), and random amplified polymorphic DNA analysis (RAPD) (2). We chose phage typing as an established phenotypic method, and PFGE and RAPD as molecular methods for the analysis of isolates from Osaka City. The effectiveness of these three methods is also discussed.

E. coli O157 strains isolated from 185 individuals including 47 healthy carriers in Osaka City from January to December 1996 were examined. Of the 138 patients, 98 (71%) reported that their symptoms started in July. Shiga toxin (Stx) production was tested by reversed-passive latex agglutination with the Verotox F kit (Denka Seiken Co., Ltd., Tokyo) according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification was used to test for the presence of *stx* genes. Two sets of primers (Cat. No. S006 and S007, Takara Co., Otsu) were used according to the manufacturer's instructions to distinguish *stx1* and *stx2*.

Isolates were phage-typed in the Laboratory of Enteric Pathogens, Central Public Health Laboratory, London, England, using the extended scheme of Khakhria et al., comprising 16 typing phages (6). The scheme recognizes 90 phage types (PTs) (personal communication R. Khakhria).

PFGE was performed by the method of Izumiya et al. (7,8) at the National Institute of Infectious Diseases, Tokyo (NIID). *Xba*I was used as the restriction endonuclease. DNA banding patterns of PFGE were classified as described by Izumiya et al. (7), according to the criteria of NIID. In brief, each DNA pattern was classified into three parts depending on the size of the DNA bands, then the ladder included in each part was analyzed in comparison with that in the corresponding part of standard strains designated by NIID. Patterns that did not correspond to the standards in three or more bands were classified as novel patterns. RAPD was performed by the method of Watanabe et al. (2). Classification of the patterns was performed according to the criteria of NIID (Manual for detection and analysis of EHEC O157, provided at a technical seminar given by NIID on 12 May 1997).

Isolates were assigned to 10 different PTs (PT-1, 2, 4, 8, 14, 21, 23, 31, 32, 34). PT-32 was the predominant type (111/185; 60%), followed by PT-8 (21/185; 11%), PT-21 (11/185; 6%), PT-4 (8/185; 4%), PT-14 (6/185; 3%), PT-1 (5/185; 3%), PT-34 (4/185; 2%), PT-31 (2/185), PT-2 (1/185), and PT-23 (1/185). Fifteen strains reacted with the phages but did not

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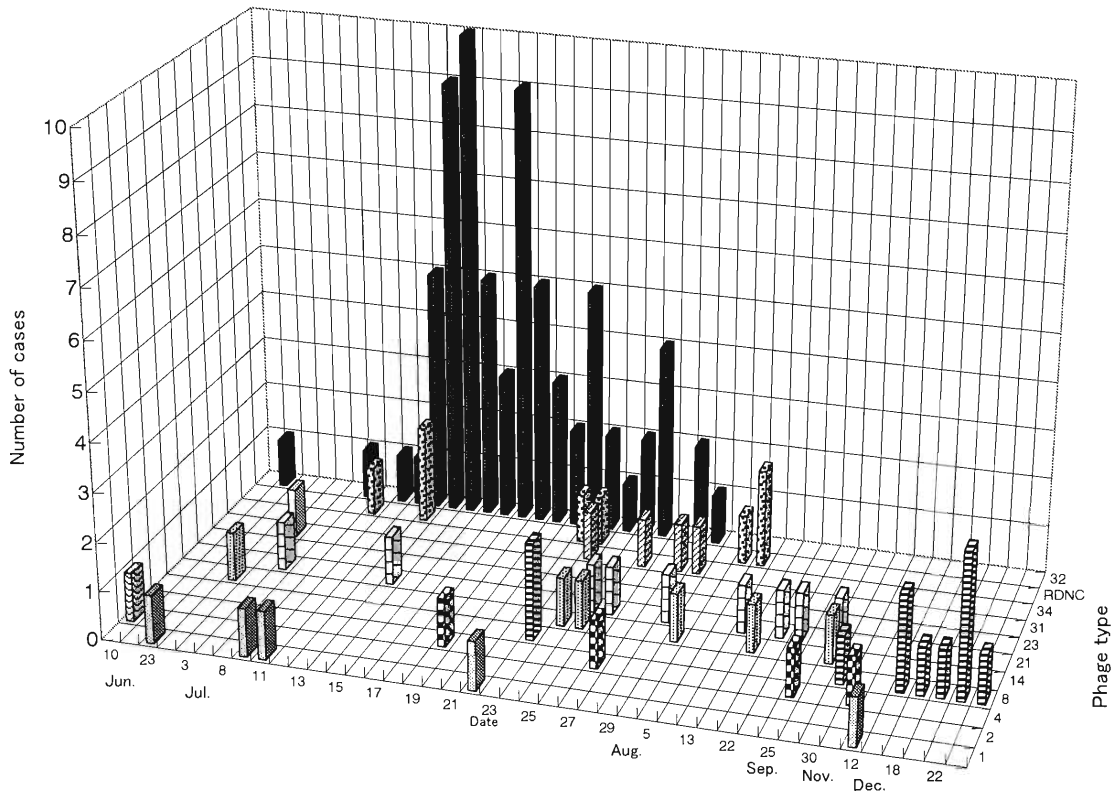

Fig. 1. Date of onset of confirmed cases and the phage type of each isolate. Each PT-32 strain, isolated from patients who became ill on 18 June, 14, or 28 July, was distinguished from the dominant type based on the PFGE patterns.

conform to a recognized type (RDNC). Eight different patterns were recognized among the 15 RDNC strains. The date of onset of patients with the prevalent type of PT-32 ranged from 8 to 29 July, and showed a peak on 15 July (Fig. 1).

PFGE gave greater strain discrimination than any other method employed, and the 185 strains showed 33 different PFGE patterns. However, some isolates showing the same PFGE pattern were divided into several different PTs, and vice versa (Table 1). Thus, by combining PFGE patterns and phage typing, isolates were classified into 42 different groups. One hundred and five isolates (57%), all of which produced both Stx1 and Stx2, belonged to PT-32 and gave the same PFGE pattern (IIa IIb I); this type was the same strain as that in the outbreak in Sakai. However, six additional PT-32 strains were distinguished from the dominant type by their PFGE patterns. The first PT-32 strain, which was isolated from a patient who became ill on 18 June (Fig. 1), gave a pattern (CX1 IIb CX1) different from that of the 105 PT-32 isolates. Similarly, two strains, isolated from patients whose symptoms started on 14 and 28 July, respectively, were also distinguished from the dominant type by PFGE. The other three strains were from healthy carriers. Seventeen strains belonging to PT-8 were isolated from patients or healthy carriers in an outbreak that occurred in a day-care center in December (Fig. 1). These strains were distinguishable by PFGE from the other four strains of PT-8 isolated during July and August.

The 185 isolates were assigned to five types (I, IIe, IIf, IIj, IIm) by RAPD patterns. Although RAPD used in this study did not contribute to further discrimination among strains showing the same PFGE pattern, strains were classified into 20 different groups by combining phage typing and RAPD.

This study showed that the use of phage typing and PFGE provided good discrimination for the study of EHEC O157.

Table 1. Discrimination by phage typing of EHEC O157 isolates that possessed the same pulsed-field gel electrophoresis (PFGE) pattern

PFGE	Phage Type	RAPD	No. of isolates
Ia CX13 CX12	4	I	1
	21	I	1
IIa IIb I	1	IIc	1
	4	IIc	6
	8	IIc	1
	14	IIc	1
	32	IIc	105
	RDNC*	IIc	3
IIa IIb CX3	14	IIc	1
	32	IIc	1
IIa IIb CX4	1	IIc	2
	14	IIc	1
	21	IIc	2
IIIc CX1 CX2	34	IIj	3
	RDNC	IIj	1

*RDNC: Reacts with the phages but does not conform to a recognized type.

Although phage typing is not as discriminatory as PFGE, it is a rapid and economic technique (5), and a useful first line-screening method. In this study, phage typing led to identifying a widespread outbreak caused by EHEC O157 PT-32 in Osaka City (Fig. 1), despite the fact that most of the cases had originally been reported as sporadic cases. We suggest that fresh food rather than preserved foods may have been the vehicle, since EHEC O157 PT-32 was isolated only for 3 weeks with a peak on 15 July. This was 4 days later than the

start of the massive outbreak of EHEC O157 infections that occurred in the primary schools in Sakai City (2). If frozen foods or other items suitable for preservation were involved, cases would have appeared over a longer period in a scattered manner. However, possible food vehicles linking the outbreaks in Osaka City and Sakai City were not identified. Although we examined foods left in patients' homes, no EHEC O157 was detected.

PFGE was the most discriminatory method used in this study, though one problem with the PFGE pattern is that the interpretation of data is difficult when patterns differ by only one or two bands, which is not unusual, particularly in the analysis of closely related organisms such as strains of EHEC O157. It has been reported that genotypic turnover changed the PFGE patterns in EHEC O157, and that this phenomenon could have epidemiological and diagnostic implications (9). Barrett et al. (4) concluded that strains which differ by a single band on PFGE could not be classified as epidemiologically related or unrelated solely on the basis of PFGE pattern. Although the use of more than one restriction enzyme for PFGE analysis has been recommended, for purposes of understanding epidemiological studies, it is useful to interpret PFGE patterns in conjunction with phage typing data provided as the first line screening (4,7). The use of both phage typing and PFGE enhances the surveillance and outbreak investigation of EHEC O157 (4,5,7,10).

Izumiya et al. reported that the strains isolated from ten outbreaks in the Kinki region (mid-western region of Japan) were similar to the Sakai type based on the PFGE pattern and phage typing data, and these outbreaks should be considered as a single outbreak (7). It is strongly suggested that the widespread outbreak in Osaka City caused by the prevalent type was also part of the huge regional outbreak. It is most likely that a significant number of sporadic cases were part of the regional outbreak not only in Osaka City but also in other cities in the Kinki region. A case-control study by the Ministry of Health and Welfare of Japan showed that the Sakai outbreak was associated with the consumption of radish sprouts (11), although EHEC O157 was not detected in sampled foods or environmental specimens. This is a plausible explanation, given that sprouts have often been a source of food-borne salmonella outbreaks (12,13).

In Osaka City, most cases were considered to have been sporadic because the attack rates were low, possibly due to low-level or sporadic contamination of food. However, phage typing clearly showed the presence of temporal clustering of a PT-32 clone of EHEC O157, suggesting a widespread outbreak. Similar situations have occurred worldwide, and are expected to increase (14). Although epidemiological methods are more sensitive than bacteriological methods for identifying the source of widespread or so-called diffuse outbreaks, such events cannot be detected if epidemiological analyses are not performed on "sporadic" cases. It is strongly suggested that epidemiological investigations are therefore essential to link together clusters of cases that at first may not appear to be related. To this end, a combination of phage typing and genotypic methods provides the optimum approach.

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