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Molecular Typing of Enterohemorrhagic *Escherichia coli* O157:H7 Isolates in Japan by Using Pulsed-Field Gel Electrophoresis

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Pulsed-field gel electrophoresis (PFGE) was applied for molecular typing of 825 enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 isolates, most of which were from 19 outbreaks and 608 sporadic cases in Japan, mainly in May to August 1996. By PFGE, the EHEC O157:H7 isolates were classified into six types (type I to V and ND [nondescript]) and UT untypeable isolates. Fifty isolates from seven outbreaks in May to June and 60 isolates from patients with sporadic cases of infection showed almost identical PFGE patterns which differed in only 1 of 22 DNA fragments. They were classified into type I. Ninety-nine isolates from 10 other outbreaks and 156 isolates from patients in the Kinki area with sporadic cases of infection obtained in the early summer of 1996 showed identical PFGE patterns, suggesting that they were derived from one huge outbreak. They were classified into type II. Type IV EHEC isolates, which had only the *stx2* gene, caused another outbreak in a primary school in June. EHEC isolates of two other types, types III and V, were not related to the outbreak but were isolated in several parts of Japan. ND EHEC isolates included a variety of patterns which could not be classified into either of the types mentioned above. Twenty-five isolates could not be analyzed due to degradation of their genomic DNAs and were represented as UT. These results indicate that EHEC O157:H7 strains with various PFGE types have already spread to Japan and caused the multiple outbreaks and sporadic infections in Japan in the summer of 1996.

Enterohemorrhagic *Escherichia coli* (EHEC) (or Shiga toxin-producing *Escherichia coli*) O157:H7 is an important foodborne pathogen causing abdominal cramps, diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) (6). Recently, EHEC O157:H7 infection has frequently been reported and has been epidemiologically linked to undercooked ground beef (5), water (11), and other foods (1, 2, 4). In Japan, an EHEC O157:H7 outbreak occurred in a kindergarten in Urawa City, Saitama Prefecture, in 1990, and 2 of 268 patients died of HUS. This outbreak was attributed to well water contaminated with EHEC O157:H7.

In May 1996, an outbreak of EHEC O157:H7 occurred in primary schools in Oku City, Okayama Prefecture, and involved 138 symptomatic patients, 2 of whom died of HUS. Eight outbreaks of EHEC O157:H7 followed in Gifu, Hiroshima, Aichi, Fukuoka, Okayama (Niimi City), and Osaka (Kawachinagano City) prefectures, Tokyo Metropolitan City, and Gunma Prefecture within 5 weeks of the first outbreak (12, 13). In addition, an extraordinarily large outbreak of EHEC O157:H7 occurred at primary schools in Sakai City, Osaka Prefecture, in July 1996. The number of symptomatic patients in Sakai City expanded to more than 5,000, and 3 of them died (13). To investigate the relationship(s) between each EHEC O157:H7 isolate from these multiple outbreaks in Japan from May to August 1996, outbreak-derived EHEC O157:H7 isolates were analyzed by pulsed-field gel electrophoresis (PFGE) (1, 8). In addition, sporadic, food, and environmental isolates obtained from May to August 1996, isolates from past outbreaks, and isolates obtained in the United States were compared to the outbreak isolates. A part of our study has been reported previously as a short letter (16). Here we describe our results of the molecular typing of EHEC O157:H7 isolates in Japan in more detail.

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MATERIALS AND METHODS

Strains. A total of 825 EHEC O157:H7 isolates, described in Table 1, were analyzed; 814 of these were isolated in Japan and the remaining 11 isolates were from the United States and were kindly provided by the Centers for Disease Control and Prevention. The Japanese isolates included 766 isolates from patients (158 isolates from 19 outbreaks and 608 from patients with sporadic cases of infection), 18 from foods, 20 from cattle feces, and 10 from various environments, including a slaughterhouse. The 11 U.S. isolates included 7 from patients; 2 from foods, 1 of which was from hamburger meat involved in the outbreak in 1993 (5); and 2 from cattle.

***stx* profile.** The presence of the *stx* genes in the EHEC O157:H7 isolates was investigated by PCR as described previously (10).

PFGE. PFGE was performed as described elsewhere (1), with minor modifications. In brief, bacterial cells on an agar medium were directly embedded in low-melting-temperature agarose (Bio-Rad Laboratories, Richmond, Calif.). After appropriate preparations for restriction endonuclease digestion were made, the DNAs in each plug were digested with 30 U of *Xba*I (Boehringer Mannheim, Mannheim, Germany) at 37°C for 4 h. PFGE was performed with a 1% agarose gel by using a CHEF DRII apparatus (Bio-Rad Laboratories) in 0.5× TBE (Tris-borate-EDTA) buffer at 10°C at 200 V. For separation of a whole genome, a linearly ramped switching time from 4 to 8 s was applied for 11 h and then a linearly ramped switching time from 8 to 50 s was applied for 9 h. For separation of fragments of less than 100 kb, a constant 4-s switching time was applied for 20 h. After PFGE, the gels were stained with ethidium bromide (0.2 μg/ml) and were photographed under UV transillumination.

Plasmid profile and its Southern blot analysis. The plasmids of the EHEC O157:H7 isolates were extracted by the modified methods of Kado and Liu (9) and Toranzo et al. (15) and were separated by conventional 0.8% agarose gel electrophoresis. For Southern blot analysis, the plasmids were transferred to a Hybond-N^+ membrane (Amersham, Little Chalfont, Buckinghamshire, England) and were probed with DNA fragments extracted from PFGE gels (see below). The probes were labeled with DIG-High Prime (Boehringer Mannheim). Detection procedures were performed by following the directions of the manufacturer.

TABLE 1. Results of the typing of EHEC O157:H7

Origin[a]	No. of isolates analyzed	*stx1*	*stx2*	PFGE pattern	Date	Source
JAPAN (814)						
Patients (766)						
Outbreaks (158 samples)						
Hiroshima	8	+	+	Ia	11 Jun 1996	Primary school
Fukuoka	5	+	+	Ia	8 Jun 1996	Day-care center
Gifu	5	+	+	Ib	7 Jun 1996	Primary school
Okayama (Niimi)	8	+	+	Ib	16 Jun 1996	Primary school
Aichi	5	+	+	Ib	7 Jun 1996	Camp school
Osaka (Kawachinagano)	8	+	+	Ib	17 Jun 1996	Primary school
Okayama (Oku)	11	+	+	Ic	28 May 1996	Primary school
Osaka (Sakai)	35	+	+	IIa	11 Jul 1996	Primary school
Osaka (Habikono)	6	+	+	IIa	15 Jul 1996	Old-age home
Osaka (Chuo-ku, Osaka)	13	+	+	IIa	13 Jul 1996	Hospital
Osaka (Higashisumiyoshi-ku, Osaka)	11	+	+	IIa	14 Jul 1996	Day-care center
Kyoto	6	+	+	IIa	16 Jul 1996	Restaurant
Wakayama (Kushimoto-1)	9	+	+	IIa	Jul 1996	Old-age home
Wakayama (Kushimoto-2)	2	+	+	IIa	Jul 1996	Old-age home
Wakayama (Hashimoto)	5	+	+	IIa	16 Jul 1996	Old-age home
Wakayama (Gobo-1)	8	+	+	IIa	17 Jul 1996	Old-age home
Wakayama (Gobo-2)	4	+	+	IIa	4 Aug 1996	Old-age home
Tokyo	4	+	+	IId	16 Jun 1996	Box lunch
Gunma	5	−	+	IV	29 Jun 1996	Primary school
Sporadic cases (608)						
	39	+, 37; −, 2[b]	+	Ia	Jun–Aug 1996	
	11	+	+	Ib	Jun–Aug 1996	
	10	+	+	Ic	Jun–Aug 1996	
	273	+	+	IIa	1990–1996	
	1	−	−	IIa	Jul 1996	
	8	+	+	IIb	1992–1996	
	2	+	+	IIc	1993, 1996	
	2	+	+	IId	1995, 1996	
	1	+	+	IIe	1992	
	4	+	+	IIg	1992–1996	
	3	+	+	IIh	Jan, Jul 1996	
	4	+	+	IIk	Jul, Aug 1992	
	13	+, 4; −, 9	+	IIIa	1991–1996	
	5	+, 2; −, 3	+	IIIb	1993–1996	
	29	+, 2; −, 27	+	IIIc	1996	
	1	−	+	IIId	Jul 1996	
	1	+	+	IIIe	Jul 1995	
	4	−	+	IIIf	Jun, Jul 1996	
	11	−	+	IV	1993, 1996	
	3	−	+	Va	Jun, Jul 1996	
	1	−	+	Vb	Jul 1996	
	102	+	+	ND	1990–1996	
	2	+	−	ND	Aug 1996	
	56	−	+	ND	1990–1996	
	2	−	−	ND	1993, 1996	
	18	+	+	UT	1993–1996	
	1	−	+	UT	Oct 1995	
	1	−	−	UT	Oct 1991	
Cattle feces (20)	1	+	+	Ic	Aug 1996	
	1	+	+	IIb	1993	
	2	+	+	IIf	1993	
	5	−	+	IIIa	1987, 1993, 1996	
	2	+	−	IIIb	Jul 1996	
	1	−	+	IIIc	1996	
	1	+	+	ND	1993	
	1	+	−	ND	1996	
	3	−	+	ND	Aug 1996	
	3	+	+	UT	1996	

Continued on following page

TABLE 1—*Continued*

Origin[a]	No. of isolates analyzed	*stx1*	*stx2*	PFGE pattern	Date	Source
Foods (18)	1[c]	+	+	Ib	Jun 1996	
	6[d]	+	+	IIa	1996	
	1	+	+	IIf	1994	
	1	−	+	IIIa	1994	
	1	−	+	IIIb	1994	
	1	−	+	IIId	1992	
	3	−	+	Va	1996	
	1	+	+	ND	Aug 1996	
	1	−	+	ND	1996	
	2	+	+	UT	1996	
Environment (10)						
Restaurants (2)	2[d]	+	+	IIa	Jul, Aug 1996	
Slaughterhouse (6)	6	+	+	IIa	1996	
Other (2)	2[d]	+	+	IIa	1996	
United States (11)						
Patients (7)	2	+	+	IIe	1993	
	1	+	+	IIg	1993	
	1	+	+	III	1993	
	1	+	+	IIIa	1993	
	2	+	+	ND	1993	
Foods (2)	1	+	+	IIa	1993	
	1	+	+	ND	1993	
Cattles (2)	1	+	+	IIc	1993	
	1	−	+	IIe	1993	

[a] Numbers in parentheses indicate total number of each group.
[b] Numbers indicate numbers of isolates.
[c] Isolate from the salad in Gifu.
[d] Containing isolates from Ehime.

RESULTS

Profiles of the *stx* genes. A total of 676 of the 825 EHEC O157:H7 isolates had both the *stx1* and the *stx2* genes; 140 had only the *stx2* gene and 5 had only *stx1* gene. Four isolates had neither gene, which might be due to their genetic instability because the *stx* genes are located in bacteriophages (14). A total of 153 isolates from 18 outbreaks in Hiroshima, Fukuoka, Gifu, Aichi, Okayama (Oku and Niimi Cities), Osaka (Kawachinagano, Sakai, Habikino, Higashisumiyoshi-ku Osaka, and Chuo-ku Osaka cities), Kyoto and Wakayama (Hashimoto, Gobo [two locations], and Kushimoto [two locations] cities) prefectures, and Tokyo Metropolitan City in 1996 had both *stx1* and *stx2*, while all 5 isolates from an outbreak in Gunma had *stx2* only (Table 1).

PFGE analysis. By using the previously reported switching times for PFGE (1), DNA bands of less than 100 kb in size could not be separated well. We adapted the switching time to obtain good resolution of fragments in this size range, as described in Materials and Methods. As a result, we found that the differences in the *Xba*I PFGE patterns of our isolates were prominent among bands of less than 100 kb (Fig. 1a), although some differences in the whole patterns were observed (Fig. 1b). According to the differences in the patterns, EHEC O157:H7 isolates were classified into six different types (types I to V and ND [nondescript]) (Fig. 1a and b). ND means that the patterns were too varied to be classified into type I to V. More than three bands of less than 100 kb were different between any two types of types I to V (Fig. 1a). Furthermore, an apparently bright band(s) was observed in each lane in Fig. 1a, for example, a 68-kb band in lane 1 and a 61-kb band in lane 2, which were helpful for characterizing the types. A total of 112 EHEC O157:H7 isolates belonged to type I, 427 belonged to type II, 66 belonged to type III, 16 belonged to type IV, 7 belonged to

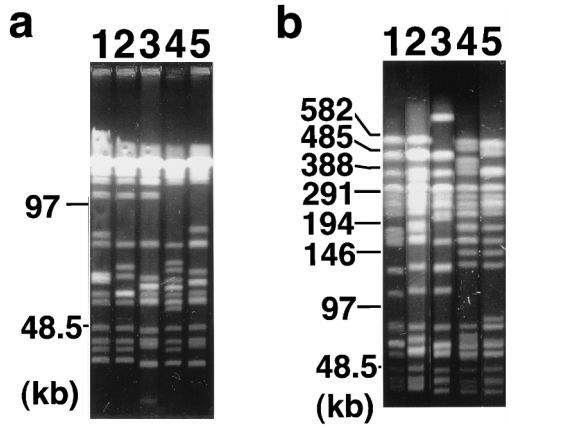

FIG. 1. (a) Representative PFGE patterns of EHEC O157:H7 isolates showing separation of fragments of less than 100 kb. Lane 1, type Ic isolate from an outbreak in Oku City, Okayama Prefecture; lane 2, type IIa isolate from an outbreak in Sakai City, Osaka Prefecture; lane 3, type IIIa isolate from cattle feces; lane 4, type IV isolate from an outbreak in Gunma Prefecture; lane 5, type Va isolate from a patient with a sporadic case of infection in Kanagawa Prefecture (see text). (b) Separation of whole genome of EHEC O157:H7 isolates. The origin of each isolate is the same as that described for panel a. The sizes of the markers are indicated to the left of each panel.

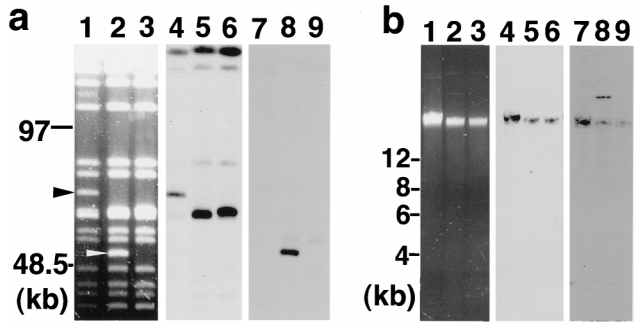

FIG. 2. (a) PFGE and Southern blot analysis results for type I strains. Lanes 1 to 3, PFGE patterns for fragments of less than 100 kb for three isolates from the Hiroshima (type Ia), Gifu (type Ib), and Okayama (Oku City) (type Ic) outbreaks, respectively. The isolates in lanes 4 to 6 and 7 to 9 are the same as those in lanes 1 to 3, respectively. A black arrowhead indicates the 75-kb fragment, and a white arrowhead indicates the 50-kb fragment. Lanes 4 to 6, results of Southern blot analysis with the 75-kb fragment as a probe; lanes 7 to 9, results of Southern blot analysis with the 50-kb fragment as a probe. (b) Results of Southern blot analysis by conventional agarose gel electrophoresis. Isolates in lanes 1 to 3, 4 to 6 and 7 to 9 are the same as those described for lanes 1 to 3 of panel a, respectively. Lanes 1 to 3, the separation of the chromosome and plasmids of each isolate; lanes 4 to 6, results of Southern blot analysis with the 75-kb fragment as a probe; lanes 7 to 9, results of Southern blot analysis with the 50-kb fragment as a probe.

type V, and 172 isolates were classified as ND (Table 1). Twenty-five isolates could not be subjected to PFGE analysis because their DNAs degraded easily, despite repeated experiments. They are represented as untypeable (UT).

All isolates from seven outbreaks which occurred in May and June 1996 (Okayama [Oku and Niimi cities], Gifu, Hiroshima, Aichi, Fukuoka, and Osaka [Kawachinagano City] prefectures) could be classified into type I. The PFGE patterns of isolates from within each outbreak were identical and differed by only one or two bands from outbreak to outbreak; a total of three patterns, named Ia, Ib, and Ic, were observed in the outbreak isolates (Fig. 2a and Table 1). In addition, 60 sporadic EHEC O157:H7 isolates obtained from June to August showed the same PFGE patterns as those of the type I outbreak isolates (Table 1; PFGE data not shown). The sizes of the different bands in patterns Ia, Ib, and Ic were 75 and 50 kb. To elucidate the origins of these two bands, i.e., plasmid or chromosome, each band was used as a probe for Southern blot analysis. At first, PFGE-separated DNA fragments were probed with the 75- or the 50-kb fragment (Fig. 2a). The 75-kb fragment hybridized to different-sized bands of the three patterns, while the 50-kb band hybridized only to itself. When probed with chromosomes and plasmids, which were separated by conventional agarose gel electrophoresis, the 75-kb fragment hybridized to the chromosomes but the 50-kb fragment hybridized to a plasmid (Fig. 2b). These results indicate that the sequence of the 75-kb fragment exists on different-sized *Xba*I fragments of the chromosomes of the type I isolates, while the 50-kb fragment is derived from a plasmid. When a variation derived from the 50-kb plasmid fragment is ignored, the 75-kb fragment in type Ia and its corresponding fragments in types Ib and Ic (Fig. 2a and Table 1) are responsible for the three PFGE patterns within type I isolates. The cause(s) of the generation of these fragments of various sizes is unknown.

The PFGE patterns of outbreak isolates from Sakai City, Osaka Prefecture, were different from those of type I isolates in more than six bands as a whole (Fig. 1b, lanes 1 and 2), three of which were less than 100 kb in size (Fig. 1a, lanes 1 and 2). These isolates were classified as type II. Although the PFGE patterns of 26 of 35 Sakai isolates analyzed so far were identical (Fig. 3a, lane 1), differences in four bands of 55, 90, 200, and 420 kb were observed (Fig. 3a). The observed frequency of the patterns shown in Fig. 3a in lanes 2 to 6 were 2 of 35, 4 of 35, 1 of 35, 1 of 35, and 1 of 35, respectively. To elucidate the origin of the 55- and the 90-kb fragments, Southern blot analysis similar to that used for type I isolates was performed by using each fragment as a probe. Both of them hybridized to plasmid bands, indicating their plasmid origin (Fig. 3b). Thus, when the plasmid bands were ignored, EHEC isolates from the Sakai outbreak showed two variations (Fig. 3a, lanes 5 and 6), both of which differed from the most general pattern (Fig. 3a, lane 1) by one band (200 or 420 kb). This suggests that the outbreak in Sakai City, Osaka Prefecture, was caused by a clonal EHEC O157:H7 strain. During the same period, nine other outbreaks occurred in Osaka (Habikino, Higashisumiyoshi-ku Osaka, and Chuo-ku Osaka Cities), Kyoto and Wakayama (Hashimoto, Gobo [two locations] and Kushimoto [two locations] cities) prefectures, which are located in the Kinki area. Furthermore, a number of sporadic EHEC O157:H7 infections in this area were reported during and after the Sakai outbreak. The PFGE patterns of 64 EHEC O157:H7 isolates from these nine outbreaks (Table 1) were the same as the most general pattern of the Sakai isolates. The PFGE patterns of 156 of 208 seemingly sporadic isolates obtained from 11 July to 4 August, that is, from the onset of the outbreak in Sakai City to the onset of the outbreak in Gobo City (Gobo-2 in Table 1), were the same as the most general pattern of the Sakai isolates as well. One food and two environmental isolates from Ehime Prefecture, which is located outside of the Kinki area, had the same *Xba*I PFGE pattern as the Sakai isolate. No epidemiological relationships between the Ehime isolates and the outbreak in Sakai City have been implicated yet. Although five other isolates from foods, eight environmental isolates in Japan, and one U.S. isolate from food were classified as type IIa, their PFGE patterns for fragments larger than 100 kb were different from those of Sakai isolates (Table 1; PFGE data not shown).

Other type II isolates included 32 Japanese isolates (4 from an outbreak in Tokyo Metropolitan City, 24 from sporadic cases, 3 from cattle feces, and 1 from food) and 5 U.S. isolates (3 from patients and 2 from cattle). They showed PFGE pat-

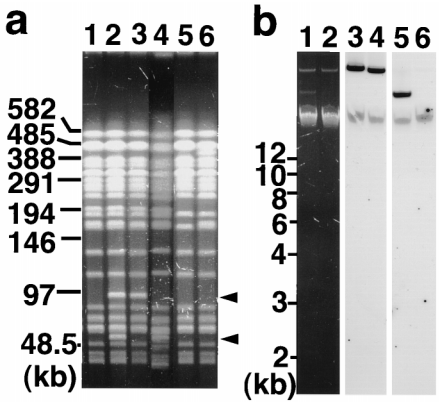

FIG. 3. (a) Six PFGE variations observed in Sakai outbreak isolates. The upper and lower arrowheads indicate 90- and 55-kb fragments, respectively. (b) Results of Southern blot analysis of conventional agarose gel electrophoresis. Isolates in lanes 1, 3, and 5 are the same as that in lane 2 of panel a, and isolates in lanes 2, 4, and 6 are the same as that in lane 3 of panel a. Lanes 3 and 4, results of Southern blot analysis with the 90-kb fragment as a probe; lanes 5 and 6, results of Southern blot analysis with the 55-kb fragment as a probe.

terns that were similar to but different from those of Sakai isolates. They showed minor banding variations for fragments of less than 100 kb, variations which were represented by subtypes (Table 1; PFGE data not shown).

Fifty-three sporadic isolates, eight isolates from cattle feces, and three isolates from food were classified into type III (Fig. 1a and b, lanes 3). Two U.S. isolates (of patient origin) were also classified into type III (Table 1).

Five isolates from an outbreak in Gunma Prefecture were classified into type IV (Fig. 1a and b, lanes 4). The same pattern as those observed for the outbreak isolates was also observed for 11 sporadic isolates from different prefectures. It remains unknown whether there is an epidemiological relationship(s) between the Gunma outbreak and these sporadic isolates.

A sporadic case of infection occurred in Kanagawa Prefecture in June 1996 and was attributed to contaminated raw cow liver, because the PFGE patterns of isolates from the patient and the liver were identical. These EHEC isolates (referred as Va in Table 1) were classified into type V (Fig. 1a and b, lanes 5).

Twenty-five isolates (20 from sporadic cases, 3 from cattle feces, and 2 from foods) could not be characterized due to degradation of genomic DNAs (represented as UT in Table 1). A total of 172 isolates (162 from patients with sporadic cases of infection, 5 from cattle feces, and 2 from foods in Japan, 2 from patients and one from food in the United States) did not show any of the patterns characteristic of types I to V. Most of them showed different patterns from each other. These isolates were named ND.

DISCUSSION

In this study, we compared EHEC O157:H7 isolates from 19 outbreaks, patients with sporadic cases of infection, various environments, and cattle by *stx* gene profiles and *Xba*I PFGE patterns (Table 1). The *stx* genes encode members of the Shiga toxin family (3). It contains two immunologically distinct group, Stx1 and Stx2 (7, 14). Stx1 is almost identical to Shiga toxin of *Shigella dysenteriae* type 1. Stx2 has about 60% homology with Stx1 and consists of several variants. Both toxins have cytotoxic activity which results in the inhibition of protein synthesis of host cells, which is considered the cause of HUS. Therefore, Stx is important for the pathogenicity of EHEC, and its production is regarded as a marker of EHEC. The profiles of *stx* revealed that most isolates in Japan had both *stx1* and *stx2* (666 of 814 isolates; 82%) and some had only *stx2* (139 of 814; 17%).

PFGE has become a standard technique for typing EHEC O157:H7 strains (1, 8). When digested with restriction endonuclease *Xba*I, genomes of EHEC O157:H7 produce more than 20 fragments ranging from 20 to 700 kb; these are suitable for separation by PFGE and provide enough information for comparing EHEC O157:H7 isolates to each other. To obtain sufficient information for the comparison, it is important to adapt PFGE conditions for separating as many fragments as possible. We modified previously described switching times (linearly ramped from 5 to 50 s) (3) in order to obtain a good resolution of the bands of less than 100 kb without a loss of separation of fragments of other sizes. The characteristic banding patterns observed in the less than 100-kb DNA bands of EHEC O157:H7 isolates were useful for classifying the isolates into five main types (types I to V) (Fig. 1 and Table 1).

We experienced 19 EHEC O157:H7 outbreaks in Japan from May to August 1996. Isolation of this organism from food, however, was very difficult; only in the outbreak in Gifu Prefecture was an EHEC O157:H7 organism isolated (from bonito salad) (13). The patient isolate from the outbreak in Gifu Prefecture and the food isolate showed identical PFGE patterns. As summarized in Table 1, our molecular analysis data indicated that the outbreaks in Hiroshima and Fukuoka prefectures were caused by EHEC O157:H7 isolates with the same genomic patterns (type Ia) and that the outbreaks in Gifu, Okayama (Niimi City), Aichi, and Osaka (Kawachinagano City) prefectures were caused by another EHEC clone (type Ib) which is very closely related to the Hiroshima-Fukuoka isolates. In addition, the third clone (type Ic), which is also related to both the Hiroshima-Fukuoka isolates and the Gifu-Okayama-Aichi-Osaka isolates, caused an outbreak in a different city (Niimi City) in Okayama Prefecture. Furthermore, multiple sporadic isolates in 18 prefectures (Japan has 46 prefectures and one metropolitan city [Tokyo]) also showed the same PFGE patterns (patterns Ia, Ib, and Ic) as those of the outbreak isolates (Table 1). Dates of isolation of the type I sporadic isolates ranged from June to August, while the outbreaks which were caused by the type I isolates ended in June. Epidemiological studies are being undertaken by each prefectural government office, but no candidate as a source of contamination has yet been implicated.

Four outbreaks in Osaka Prefecture (Sakai, Habikino, Chuo-ku Osaka, and Higashisumiyoshi-ku Osaka cities), one in Kyoto Prefecture, and five in Wakayama Prefecture (one in Hashimoto, two in Kushimoto, and two in Gobo cities) occurred during the same period. The isolates from those outbreaks and most of the sporadic isolates obtained during this period showed the same PFGE patterns, suggesting that the 10 outbreaks described above and seemingly sporadic isolates were part of one huge outbreak. 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 (12). Epidemiological studies on the relatedness of the isolates from the other nine outbreaks and the sporadic isolates with the isolates from the Sakai outbreak are required.

In the autumn of 1996, Japan had an additional three outbreaks, in a primary school in Iwate Prefecture and in a day-care center in Saga Prefecture in September and in a kindergarten in Hokkaido Prefecture in October. The PFGE patterns of the Iwate isolates were classified into type II, which is not identical to that of the Sakai isolates (data not shown). The Saga isolates showed the same PFGE pattern as the isolates responsible for the Gunma outbreak in June (data not shown). The PFGE patterns of the Hokkaido isolates were classified into type III (data not shown). Epidemiological studies are being undertaken by each prefectural government office.

This study showed that various genotypes of EHEC O157:H7 have spread throughout Japan. To prevent further spreading of contaminated food throughout Japan, coordination of field and molecular epidemiological studies of EHEC O157:H7 outbreaks are essential. For this reason, we plan to incorporate our molecular epidemiological analysis method into the surveillance systems for EHEC outbreaks in Japan.

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