Epidemiol. Infect. (2000), **125**, 221–224. Printed in the United Kingdom

SHORT PAPER

A comparison of *Escherichia coli* O157 isolates from cattle in Japan and the USA by molecular biological methods

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(*Accepted 27 March 2000*)

SUMMARY

Escherichia coli O157 isolates from cattle in Japan ($n = 91$) and in the USA ($n = 415$) were compared by pulsed-field gel electrophoresis of endonuclease-cleaved genomic DNA, location of the *stx* genes and bacteriophage typing. Three isolates from cattle in Japan with high similarity to isolates from cattle in the USA were found. Isolates from cattle farms in Japan and the USA may share a common source.

Enterohaemorrhagic *Escherichia coli* O157:H7 is now recognized as one of the most important foodborne pathogens, especially in developed countries [1]. In Japan, since the first outbreak of this agent in a kindergarten in Urawa city, 1990, the number of reports of sporadic infection has been increasing slightly [2]. In 1996, more than 20 outbreaks and multiple sporadic infections of *E. coli* O157:H7 occurred at various sites in Japan [3–5]. The Ministry of Agriculture, Forestry and Fisheries of Japan conducted on-farm surveys to evaluate the sources of this agent from August to October in 1996. *E. coli* O157:H7 was isolated from 0·62% of cattle faecal samples but not from any of the samples taken from pigs [6]. A total of 77 *E. coli* O157:H7 isolates from cattle in Japan were investigated by molecular biological methods. Fifteen bacteriophage types and 50 pulsed-field gel electrophoresis (PFGE) profiles were observed [7]. These results indicate that *E. coli* O157:H7 strains with various genetic subtypes have already spread to cattle in Japan. One explanation for this diversity is that exchange of this agent between Japan and other countries has added to the background of genetic diversity produced by mutation and recombination. If so, we should find identical or closely related *E. coli* O157:H7 from cattle raised in places geographically wide apart, such as Japan and the USA. To test this hypothesis, we compared *E. coli* O157:H7 isolates from cattle in Japan with those from cattle in the USA by using molecular methods.

A total of 91 *E. coli* O157:H7 (H−) isolates from 91 cattle in Japan (Japanese isolates) were primarily obtained from a previous investigation [7]. Seventy-seven of these isolates were isolated from faecal samples from August to October in 1996 at regional livestock hygiene service centres of 23 prefectures (Japan has 46 prefectures and a capital city, Tokyo). Fourteen isolates were isolated from faecal samples from three prefectures in 1996 and 1997. A total of 415 *E. coli* O157 isolates from 415 faecal samples from

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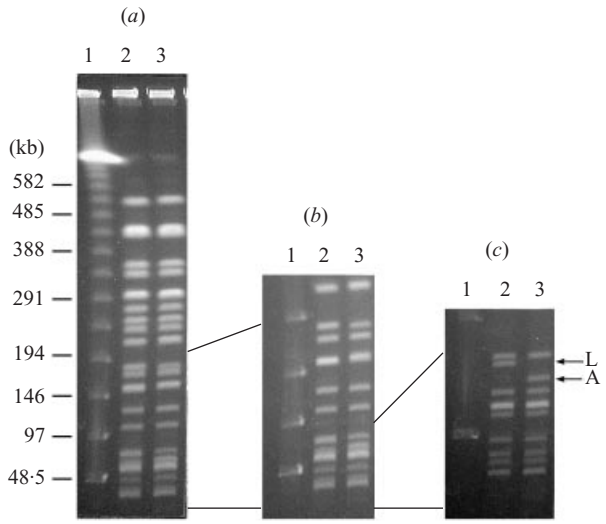

Fig. 1. Comparison of *E. coli* O157: isolates from cattle in Japan and the USA by PFGE using *Xba*I-cleaved genomic DNAs. (a) Switching times ramped from 5 to 50 s for 24 h was used to compare all sized bands. (b) Switching times ramped from 10 to 20 sec for 22 h was used to compare < 300 kb sized bands. (c) Constant switching time of 4 sec for 20 h was used to compare < 100 kb sized bands. In Fig. 1(a)–(c), lane 1 is a Lambda ladder as a size marker. Lane 2 is a profile of the Japanese isolate JP37. Lane 3 is a profile of the US isolate US1914. L indicates a lack of the band and A indicates an additional band, in the profile of the US isolate compared to that of the Japanese isolate. In this case, two band differences were determined.

cattle in the USA (US isolates) were also obtained from a previous investigation. Three hundred and three isolates were isolated from 41 different farms in the states of Washington, Oregon and Idaho during 1991–5 [8]. A hundred and twelve isolates were isolated from 31 feedlots located throughout the USA in 1994 [9]. None of these US isolates was assayed for the H flagellar antigen.

PFGE profiles using *Xba*I (Gibco–BRL, Gaithersburg, MD) digested genomic DNA were initially obtained. Genomic DNA was prepared by a previously described method [8]. Restriction endonuclease digestion was performed using 30 U of *Xba*I. PFGE was performed in 1% agarose gels using a CHEF DR II apparatus (Bio-Rad, Hercules, CA) in 0.5XTBE (Tris-borate-EDTA buffer) at 13 °C at 200 V. The pulse time was increased from 5 to 50 s over 20 h. Lambda ladders (Bio-Rad) were used as size markers. Agarose gels were stained with ethidium bromide and photographed under ultraviolet transillumination.

Each PFGE profile was compared with all of the others by a previously described method [8]. Briefly, a restriction fragment length polymorphism (RFLP) analysis software programme (DNA ProScan PRO-RFLP, Nashville, TN) was used to estimate the sizes of the largest nine bands of DNA by using scanned images of photographs. A SAS clustering procedure (SAS Institute, Cary, NC) was then used to designate the possible indistinguishable profiles, and comparison of individual isolates within clusters was performed by a spreadsheet programme that utilized the data on DNA band size. Twenty-eight of 91 Japanese isolates had identical profiles to 69 US isolates using the largest 9 bands.

*Xba*I-cleaved DNA from these isolates was compared by running them together on agarose gels using three kinds of switching times to fractionate and compare restriction fragments (Fig. 1): (i) < 600 kb, ramped from 5 to 50 s for 24 h; (ii) < 300 kb, ramped from 10 to 20 s for 22 h; and (iii) < 100 kb, constant switching time of 4 s for 20 h. The isolates having similar profiles in all sized bands were further compared by PFGE using additional restriction endonuclease *Bln*I (Boerhinger–Mannheim, Mannheim, Germany) and *Spe*I (Promega, Madison, WI). Restriction endonuclease digestion was performed using 30U of each enzyme. The pulse time was increased from 5 to 50 s over 24 h for *Bln*I–digested DNAs and 10–20 s over 22 h for *Spe*I-digested DNAs.

We also compared the toxin gene location on the PFGE profiles of *Xba*I-cleaved DNA of these isolates by southern blot analysis probed by the *stx1* and *stx2* genes. *Xba*I-cleaved DNA of these isolates were transferred to positively charged nylon membranes (Boerhinger–Mannheim) in 0.4 M NaOH solution. A 471 bp DNA fragment of the *stx1* gene and of the *stx2* gene were amplified with the primer pairs described by Kobayashi and colleagues [10], and with DNA from *E. coli* NIH 212 strain, which possesses both *stx1* and *stx2* genes. This strain, isolated from patient in the large outbreak in Sakai city, Osaka prefecture, Japan, in 1996, was kindly provided by the National Institute of Infectious Diseases of Japan. The amplified fragments were labelled with digoxigenin-11-dUTP. Probe labelling, hybridization and detection of filter-bound labelled DNA were performed according to the manufacturer's protocol (DIG DNA Labelling and Detection Kit, Boerhinger–Mannheim).

Bacteriophage typing of these isolates was performed by the methods originally described by Ahmed and colleagues [11] and extended by Khakhria and colleagues [12].

We found that 3 of 28 Japanese isolates were very

https://doi.org/10.1017/S0950268899004264 Published online by Cambridge University Press

Table 1. E. coli *O157 isolates from cattle with high similarity between Japan and the USA*

Isolates*	Origin	Date of isolation	Numbers of band differences†			Toxin gene location‡		PT§
			*Xba*I	*Bln*I	*Spe*I	*stx1*	*stx2*	
JP37	Japan	Aug. 1996	—	—	—	70	530	21
US1914	USA	Nov. 1994	2	0	4	70	530	32
JP88	Japan	Sep. 1996	—	—	—	—	130	54
US1920	USA	Nov. 1994	2	3	1	—	130	54
JP102	Japan	Jun. 1997	—	—	—	70	520	26
US1412	ID, USA	Sep. 1994	5	3	0	70	530	26
US1665	WA, USA	Oct. 1994	5	2	0	70	530	32
US1814	WA, USA	Nov. 1994	5	4	0	48	530	RDNC‖
US3220	ID, USA	Sep. 1995	4	2	1	70	530	14

* JP37 was similar to US1914. JP88 was similar to US1920. JP102 was similar to US1412, US1665, US1814 and US3220.
† Number of band of sizes different from those in Japanese isolates.
‡ Size of band where *stx* genes are located.
§ Bacteriophage types.
‖ Isolate reacts with typing phages but does not conform to a currently defined pattern.

similar to at least one of the US isolates (Table 1). The numbers of band differences between Japanese and US isolates in the PFGE profiles of *Xba*I-cleaved genomic DNAs were less than six. By *Bln*I digestion, the isolate JP37 was indistinguishable from the isolate US1914. JP102 was indistinguishable from the isolates US1412, US1665 and US1814 by *Spe*I digestion. The toxin gene location of the isolates JP37 and JP88 were the same as that of the isolates US1914 and US1920, respectively. Moreover, the phage types of the isolates JP88 and JP102 were the same as the isolates US1920 and US1412, respectively.

PFGE is widely used as a genetic subtyping method of *E. coli* O157:H7 because of its high discriminatory power and reproducibility [3, 13–17]. According to guidelines proposed by Tenover and colleagues [18], six or fewer band differences indicate a possible genetic relationship between two isolates. However, the authors restricted the use of these criteria to analysing small sets of isolates collected over relatively short periods of time in hospitals or communities. They recommended the use of multiple restriction endonucleases and other analyses in investigations for potential genetic relationships among isolates collected over extended periods. On the other hand, we observed up to four band differences from the chromosome origin between the inoculated and recovered *E. coli* O157:H7 isolates from experimentally infected cattle [19]. Up to four band differences on PFGE profiles of *E. coli* O157:H7 isolates from cattle may indicate the possibility that the isolates share the same origin.

Bacteriophage typing has a relatively high discriminatory power as a subtyping method of *E. coli* O157:H7, though it examines a phenotypic trait. Simpson's index of diversity [20] for phage types was 0·884, while that for PFGE types was 0·985 in a previous investigation. It has been suggested that combined use of bacteriophage typing and PFGE enhances reliability in molecular epidemiologic surveys [7, 21].

Because the Japanese and the US *E. coli* O157:H7 isolates had been collected over a period of 7 years and from a wide area, we compared them directly by PFGE using three kinds of endonuclease. We found that 3 of 28 Japanese isolates were very similar to US isolates (Table 1). The numbers of band differences were five or less using any enzyme. Moreover, the results from toxin gene location and bacteriophage typing suggested a close relation between the Japanese isolates and the US isolates. These Japanese and US isolates may share the same origin. Common reservoirs of colonization for cattle farms may exist in Japan and the USA.

Rice and colleagues [8] reported that identical subtypes of *E. coli* O157 isolates were found on farms separated by as much as 640 km. They suggested the possibility that *E. coli* O157:H7 can be transmitted inter-regionally to cattle farms. They also suggested that cattle feed can be a vehicle for this transmission. Although *E. coli* O157:H7 has not been reported to have been found in marketed cattle feeds, our finding of *E. coli* O157:H7 isolates with high similarity from cattle in Japan and the USA supports this possibility.

https://doi.org/10.1017/S0950268899004264 Published online by Cambridge University Press

This work was supported by a grant from the Science and Technology Agency of the Japanese government. The authors thank Drs Wendy Johnson, Rasik Khakhria and Rafiq Ahmed (Laboratory Centre for Disease Control, Health Canada, Canada) for instruction regarding the method of bacteriophage typing.

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