Epidemiol. Infect. (1999), **122**, 337–341. Printed in the United Kingdom

SHORT PAPER

Molecular typing of *Escherichia coli* O157:H7 (H−) isolates from cattle in Japan

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(*Accepted 2 December 1998*)

SUMMARY

A total of 77 *Escherichia coli* O157:H7 (H−) isolates from cattle in Japan were investigated by molecular biological methods. Most of these isolates (43 isolates) possessed the *stx2* gene, but not *stx1*. Fifteen bacteriophage types and 50 pulsed-field gel electrophoresis (PFGE) profiles were observed. One isolate was indistinguishable from the human outbreak strain by these methods. This indicates that cattle must be considered as a possible source of human *E. coli* O157:H7 infection in Japan.

Escherichia coli O157:H7 is an important foodborne pathogen which causes diarrhoea, severe abdominal cramps and haemorrhagic colitis, sometimes complicated by haemolytic uraemic syndrome [1]. This pathogen was first identified in an outbreak in the United States in 1982 [2]. In the outbreaks reported to the Centers for Disease Control and Prevention in the United States up to 1994, ground beef was identified as the vehicle of this pathogen in 58% of the foodborne outbreaks [3]. *E. coli* O157:H7 has been isolated from the faeces of 1–10% of sampled cattle in surveys in North America and Europe [3]. The agent appears to be virtually ubiquitous on cattle farms [4]. Therefore cattle are suspected to be one of the most important sources of *E. coli* O157:H7 in North America and Europe [3].

In Japan, since the first outbreak of this pathogen in a kindergarten in Urawa city, 1990, the number of isolations by prefectural and municipal public health institutes has been increasing slightly [5]. In 1996, more than 20 outbreaks and multiple sporadic infections of *E. coli* O157:H7 occurred in many places in Japan [6–8]. However, products of bovine origin have neither been confirmed nor suspected as the vehicle for *E. coli* O157:H7 outbreaks in Japan [6, 7]. Furthermore, the molecular genetic characteristics of *E. coli* O157:H7 isolates from cattle in Japan have not been established, and their genetic relationship to human isolates is therefore unclear.

The purpose of this study was to evaluate the potential of cattle as a source of the human *E. coli* O157:H7 infections in Japan. We subtyped *E. coli* O157:H7 (H−) isolates from cattle in Japan by toxin genotype, bacteriophage type and pulsed-field gel electrophoresis (PFGE), and compared these data with existing data on human isolates. This is the first report of molecular typing of *E. coli* O157:H7 (H−) isolates from cattle in Japan.

Sixty-nine *E. coli* O157:H7 isolates and 8 *E. coli* O157:H− isolates from 77 cattle faecal samples were isolated by routine diagnostic methods [9] from August to October in 1996 at regional livestock hygiene service centres of 23 prefectures in Japan.

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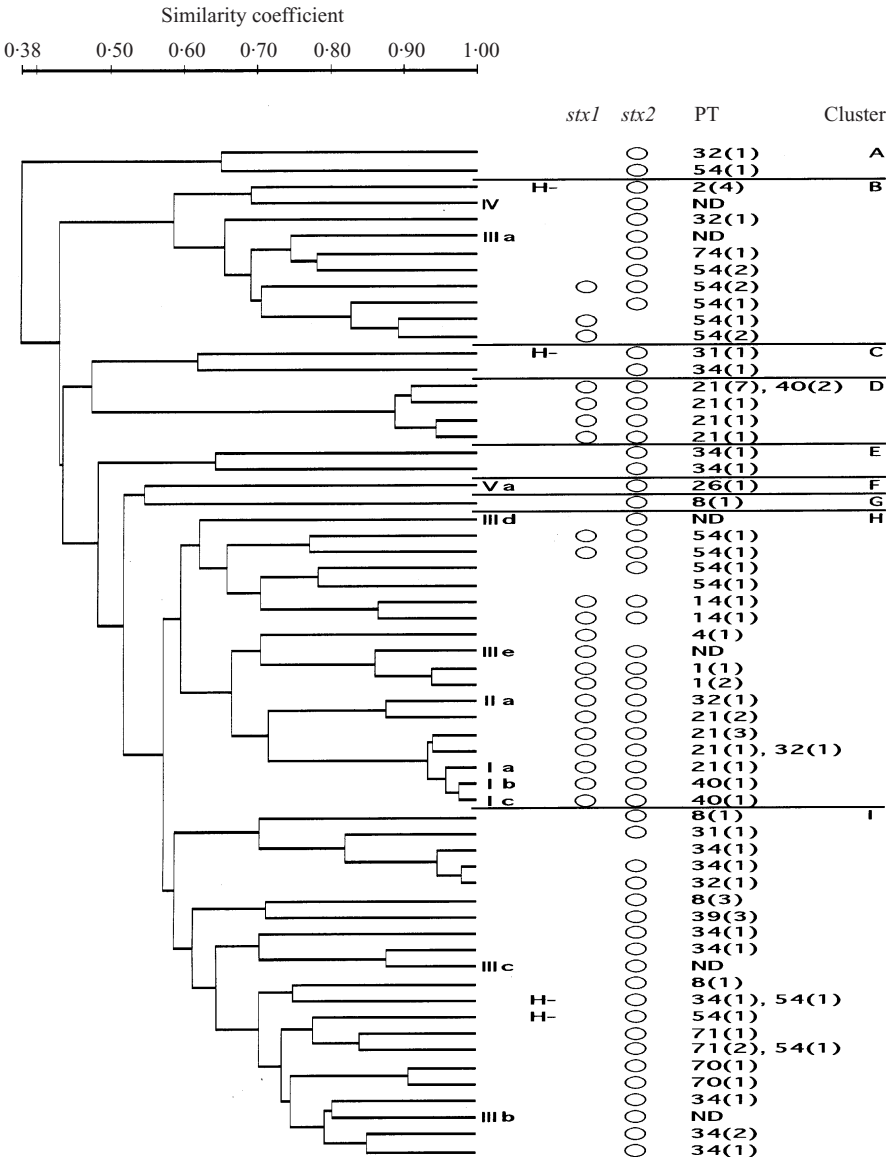

Fig. 1. Comparison of bovine and human *E. coli* O157:H7 isolates based on the cluster analysis of *Xba*I digestion profiles with the toxin genotype and bacteriophage type. Roman numerals indicate the classification of the PFGE profiles of human isolates by the National Institute of Health of Japan. H− indicates *E. coli* O157:H− isolates. PT is the abbreviation for phage type. The numbers in the parentheses indicate the number of isolates. Nine clusters were observed at a similarity coefficient of 0·58 and are designated A to I.

Eleven isolates from patients in 1996 were provided by the National Institute of Health (NIH) of Japan and were used as reference strains. These human isolates possessed a variety of PFGE patterns belonging to types Ia, Ib, Ic, IIa, IIIa, IIIb, IIIc, IIId, IIIe, IV, Va (NIH of Japan classification) [6].

The presence of the Shiga-toxin (*stx*) genes of the isolates were determined by PCR using the methods of Pollard and colleagues [10]. Among the 77 isolates from cattle, 75 isolates (97·4%) had genes for either *stx1* or *stx2*, and 28 isolates (36·4%) had genes for both *stx1 and stx2*. Forty-three isolates (55·8%) possessed the gene for *stx2* only and four isolates (5·2%) possessed the gene for *stx1* only. The remaining two isolates (2·6%) had neither *stx* gene.

Bacteriophage typing was performed by the methods originally described by Ahmed and colleagues [11] and extended by Khakhria and colleagues [12]. Fifteen phage types were observed among the cattle isolates: 1, 2, 4, 8, 14, 21, 31, 32, 34, 39, 40, 54, 70, 71 and 74 (Fig. 1). Three phage types, 21 (16 isolates), 54 (16 isolates), 34 (11 isolates) were dominant, and other page types were represented by six or fewer isolates.

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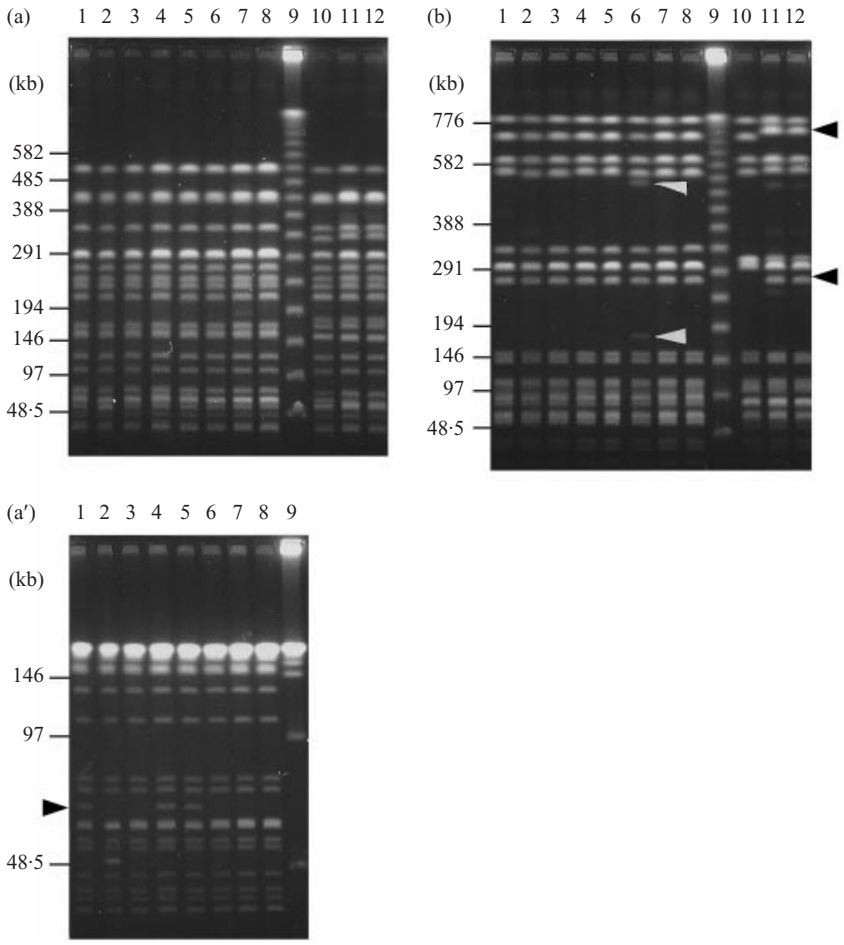

Fig. 2. (*a*, *a′*) Bovine isolates having similar *Xba*I digestion profiles to those of human isolates. Lanes 1–3 are types Ia, Ib and Ic isolates, respectively, from patients and lanes 4–8 are bovine isolates. Lane 5 and 6 are isolates from same prefecture and have a 75 kb band (arrowhead on *a′*) specific for type Ia. Lanes 6–8 isolates are from another prefecture and have same PFGE pattern for bands less than 100 kb as a type Ic human isolate. Lane 9 is a Lambda ladder as a size marker. Lane 10 is a type IIa isolate from a patient and lanes 11 and 12 are bovine isolates from one prefecture. (*b*) Discrimination of the same isolates as described for panel (*a*) by *Bln*I digestion. Type IIa isolates from cattle (lanes 11 and 12) can be clearly discriminated from the human isolate (lane 10) by the size difference of the second most largest band (upper black arrowhead) and a presence of a 280 kb band (lower black arrowhead). One of type Ic isolates (lane 6) can also be discriminated from human isolates by the presence of a 500 and a 180 kb band (white arrowheads). Remaining type I isolates from cattle (lanes 4, 5, 7, 8) can be discriminated from human isolates (lanes 1–3) by *Bln*I digestion.

PFGE profiles were obtained as follows. Genomic DNAs of each isolate were prepared by the method of Izumiya and colleagues [6]. Restriction endonuclease digestion was performed using 30 U of *Xba*I or *Bln*I (Takara Shuzo Co., Kyoto, Japan). Electrophoresis was performed in a 1% agarose gels using CHEF DRIII apparatus (Bio-Rad Laboratories, Richmond, CA, USA) in 0·5× Tris-borate-EDTA buffer at 10 °C at 200 V. The pulse time for *Xba*I was increased from 2 to 50 s for 20 h. For separation of bands less than 100 kb, a constant switching time 4 s was applied for 16 h. Pulse time for *Bln*I was increased from 2 to 50 s for 20 h. Lambda ladders (Bio-Rad Laboratories) were used as size markers.

Digestion profiles of bovine and human isolates by *Xba*I were compared with each other by using the Dice similarity coefficient and Diversity Database Software (PDI, Huntington Station, NY, USA). Cluster analysis was done using the hierarchic unweighted pair arithmetic average algorithm (maximum tolerance, 2·0%), and a dendrogram was prepared.

Using *Xba*I, 50 PFGE profiles were observed among 77 bovine isolates. Seven isolates had similar *Xba*I PFGE profiles to human isolates: 5 strains from 2 prefectures had types Ia and Ic profiles and 2 strains from 1 prefecture had a type IIa profile (Fig. 2*a* and *a′*). Four of these seven bovine isolates with types Ia and Ic profiles were indistinguishable from human

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Table 1. *Phage types of bovine isolates with similar PFGE profiles to human isolates*

PFGE type*	*stx*	Phage types of human isolates†	No. of bovine isolates	Phage types of bovine isolates
Ia	1, 2	21	2	21, 32
Ic	1, 2	40	3	21
IIa	1, 2	1, 4, 8, 14, 32	2	21

* Classification by National Institute of Health (NIH) of Japan [6].
† Data by NIH of Japan [19].

isolates using *Bln*I digestion (Fig. 2*b*). Two of the bovine isolates (type Ia) were also indistinguishable from human isolates by phage typing (Table 1).

Cluster analysis was done by using 50 *Xba*I digestion profiles from bovine isolates and 11 *Xba*I digestion profiles from human isolates. Nine clusters were observed at a similarity coefficient of 0·58. Samples with similarity coefficient of more than 0·88 were closely related (Fig. 1). Human isolates were distributed among cluster B, F, H and I (Fig. 1). All four profiles of cluster D and 15 of 18 profiles of cluster H were observed among isolates having both *stx1* and *stx2* genes. On the other hand, all the profiles of cluster A, C, E, F, G and I were observed among isolates having the *stx2* gene only or neither *stx* gene (Fig. 1). Dominant phage types 21, 54 and 34 were each distributed among two or more different clusters (Fig. 1). Isolates with indistinguishable PFGE profiles were of multiple phage types (Fig. 1).

PFGE is widely used as a molecular subtyping method of *E. coli* O157:H7 because of its high discriminatory power and good reproducibility [6, 13–17]. In this study, Simpson's index of diversity (Simpson's *D*) [18] for PFGE was 0·985. This suggests that PFGE has sufficient discriminatory power to subtype bovine *E. coli* O157:H7 isolates in Japan. In general, discriminatory power of phage typing is lower than that of subtyping by PFGE. Krause and colleagues [17] reported that the Simpson's *D* was 0·786 for phage types and 0·987 for PFGE types of *E. coli* O157:H7. In this study, the Simpson's *D* for phage types was a relatively high value of 0·884. The results of phage typing and PFGE subtyping were discordant in that each phage type was distributed throughout several PFGE defined clusters. These results suggest that combined use of bacteriophage typing and PFGE provides a more detailed classification of isolates.

Four bovine isolates were indistinguishable from human isolates by PFGE using two kind of endonuclease. One of the four isolates was also indistinguishable from human isolates by phage typing. These results strongly suggest that the bovine isolate is the same clone as the human isolates; however, there was no epidemiologic information concerning the connection between these cattle and human outbreaks. On the other hand, 12 phage types that we found among bovine isolates were also observed in human *E. coli* O157:H7 isolates in Japan [19]. These data indicate that cattle must be considered as a possible source of human *E. coli* O157:H7 infection in Japan as well as North America and Europe. Effective on-farm control measures of this pathogen are needed to prevent human *E. coli* O157:H7 infection in Japan.

This work was supported by grants from the Ministry of Agriculture, Forestry and Fisheries and the Science and Technology Agency of the Japanese Government. We gratefully acknowledge regional livestock hygiene service centres in Japan and the National Institute of Health of Japan for kindly providing *E. coli* O157:H7 (H−) isolates. We thank Drs Wendy Johnson, Rasik Khakhria and Rafiq Ahmed (Laboratory Centre for Disease Control, Health Canada, Canada) for instruction about the method of bacteriophage typing. We also thank Drs Dale Hancock, Thomas Besser, Daniel Rice, Margaret Davis and Jeffrey LeJeune (Washington State University) for helpful comments on preparing the manuscript.

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