Epidemiol. Infect. (1999), **122**, 343–346. Printed in the United Kingdom

SHORT PAPER

The shift of genetic subtypes of *Escherichia coli* O157:H7 isolates from cattle

M. AKIBA*, T. SAMESHIMA AND M. NAKAZAWA

Laboratory of Zoonosis, Feed Safety Research Division, National Institute of Animal Health, Kannondai 3-1-1, Tsukuba, Ibaraki, 305-0856, Japan

(*Accepted 8 December 1998*)

SUMMARY

A total of 46 *Escherichia coli* O157:H7 isolates were obtained from sequential faecal samples from seven cattle collected over periods of 2 months. Nine closely related genetic subtypes, determined by pulsed-field gel electrophoresis types using three kinds of restriction endonuclease were observed among the isolates. Distinguishable, but closely related genetic subtypes can be isolated from one farm, or from one cow, should be considered when undertaking an epidemiological survey.

Escherichia coli O157:H7 is recognized as an important foodborne pathogen in many countries and cattle are suspected to be one of the most important sources of this agent [1]. In the outbreaks reported to the Centers for Disease Control and Prevention in the United States up to and including 1994, ground beef was identified as the vehicle of this pathogen in 58% of the foodborne outbreaks [1]. Not only foodborne infection, but also direct transmission from cattle to humans were also reported [1–3]. In these cases, the correspondence of molecular types of isolates from cattle and humans was the primary evidence of the transmission.

Pulsed-field gel electrophoresis (PFGE) is widely used for genetic subtyping of this pathogen because of its high discriminatory power and good reproducibility [4–10]. The PFGE analysis of *E. coli* O157:H7 isolates from cattle has been reported [7, 9–11]. Most of these isolates were, however, collected at one time point and the extent of variation of PFGE patterns in the course of carriage is not clear. We attempted the sequential culture of faeces obtained from the seven naturally infected cattle on one farm and performed the genetic subtyping of the isolates based on PFGE.

Faecal samples were collected from 670 cattle of farm A in Japan on 20 September or 3 October 1996. Non-sorbitol fermenting colonies were selected by direct plating on MacConkey sorbitol agar (Difco, Detroit, MI, USA) plates with cefixime (50 ng/ml) (Dynal, Oslo, Norway) and tellurite (2·5 μg/ml (Dynal) (SMAC-CT) and further identified as *E. coli* O157:H7 by routine diagnostic methods [7]. *E. coli* O157:H7 isolates were detected from seven cattle at the time points. Three of these were 3- to 6-month-old Holsteins and four were 5- to 15-month-old Japanese Black breed. All animals were clinically normal. Sequential faecal samples of these cattle were collected every week from 30 October to 4 December 1996. Faecal culture was performed by using the following method. About 1 g of faeces were inoculated to 10 ml of broth enrichment media (mEC; Kyokuto Pharmaceutical Co., Tokyo, Japan) with novobiocin (20 μg/ml) (Sigma Chemical Co., St Louis, MO, USA) and incubated for 20 h at 42 °C. After concentration by immunomagnetic separation (Dynabeads [anti-*E. coli* O157:H7]; Dynal), the media were spread on SMAC-CT and incubated overnight at 37 °C. Up to 12 non-sorbitol fermenting colonies were selected and identified as *E. coli* O157:H7 by routine diagnostic methods [7].

Thirty-nine *E. coli* O157 isolates were obtained from nine positive faecal samples collected from 30 October to 20 November. Together with seven isolates

* Author for correspondence.

Table 1. *Genotypic characteristics of* E. coli *O157 : H7 isolates*

Bovine no.	Isolate	Date of isolation	Stx2	PFGE type			Genetic subtype
				*Xba*I	*Bln*I	*Spe*I	
1	1-0	3 Oct 1996	+	1	1	1	I
2	2-0	3 Oct 1996	+	1	1	1	I
3	3-0	20 Sep 1996	+	1	1	1	I
	3-1-1	30 Oct 1996	+	1	2(1)*	1	II
	3-1-2	30 Oct 1996	+	1	1	1	I
	3-1-3	30 Oct 1996	+	1	1	1	I
	3-1-4	30 Oct 1996	+	1	1	1	I
	3-1-5	30 Oct 1996	+	1	1	1	I
	3-1-6	30 Oct 1996	+	1	1	1	I
	3-1-7	30 Oct 1996	+	1	1	1	I
	3-1-8	30 Oct 1996	+	1	1	1	I
	3-1-9	30 Oct 1996	+	1	1	1	I
	3-1-10	30 Oct 1996	+	1	1	1	I
	3-1-11	30 Oct 1996	+	1	1	1	I
	3-2-1	6 Nov 1996	+	1	1	1	I
	3-2-2	6 Nov 1996	+	1	1	1	I
	3-3-1	13 Nov 1996	−	1	3(2)	2(2)	III
	3-3-2	13 Nov 1996	−	1	3	2	III
	3-3-3	13 Nov 1996	−	1	4(3)	2	IV
	3-3-4	13 Nov 1996	−	1	3	2	III
	3-3-5	13 Nov 1996	−	1	3	2	III
	3-3-6	13 Nov 1996	−	1	3	2	III
	3-3-7	13 Nov 1996	−	1	3	2	III
	3-4-1	20 Nov 1996	+	1	1	1	I
	3-4-2	20 Nov 1996	+	1	1	1	I
	3-4-3	20 Nov 1996	+	1	1	1	I
	3-4-4	20 Nov 1996	+	1	1	1	I
	3-5-1	27 Nov 1996	−	2(2)	3	2	V
	3-5-2	27 Nov 1996	−	2	5(2)	2	VI
	3-5-3	27 Nov 1996	−	2	5	2	VI
	3-5-4	27 Nov 1996	−	2	5	2	VI
4	4-0	20 Sep 1996	+	1	1	1	I
	4-4-1	20 Nov 1996	+	1	1	1	I
5	5-0	20 Sep 1996	+	1	1	1	I
6	6-0	3 Oct 1996	+	3(7)	3	3(5)	VII
	6-4-1	20 Nov 1996	+	1	1	1	I
	6-4-2	20 Nov 1996	+	1	1	1	I
	6-4-3	20 Nov 1996	+	1	1	1	I
	6-4-4	20 Nov 1996	+	1	1	1	I
7	7-0	3 Oct 1996	+	4(3)	1	4(2)	VIII
	7-2-1	6 Nov 1996	+	1	6(3)	1	IX
	7-2-2	6 Nov 1996	+	1	6	1	IX
	7-4-1	20 Nov 1996	+	1	1	1	I
	7-4-2	20 Nov 1996	+	1	1	1	I
	7-4-3	20 Nov 1996	+	1	1	1	I
	7-4-4	20 Nov 1996	+	1	1	1	I

* Number of band of sizes different from those in type 1.

from faecal samples collected on 20 September and 3 October, a total of 46 *E. coli* O157 isolates were analysed by PFGE and PCR.

All isolates were subtyped using pulsed-field gel electrophoresis patterns. Genomic DNA of each isolates were prepared by the method of Izumiya and colleagues [10] with minor modification. In brief, all isolates were grown in Lauryl-broth for 16 h at 37 °C. 100 μl of bacterial culture was centrifuged at 14000 ***g*** for 3 min, and the bacterial pellets were embedded in

https://doi.org/10.1017/S0950268899002204 Published online by Cambridge University Press

low-melting-temperature agarose (Bio-Rad Laboratories, Richmond, CA, USA). After the pellets were lysed with lysozyme and digested with proteinase K under the appropriate condition, restriction endonuclease digestion was performed with 30U of *Xba*I, *Bln*I, or *Spe*I (Takara Shuzo Co., Kyoto, Japan) at 37 °C for 4 h. Electrophoresis was performed with a 1% agarose gel using CHEF DR III apparatus (Bio-Rad Laboratories) in 0·5 × trisborate-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 of 4 s was applied for 16 h. Pulse time for *Bln*I was increased from 2 to 50 s for 20 h. And pulse time for *Spe*I was increased from 10 to 20 s for 22 h.

Among the 46 *E. coli* O157:H7 isolates, 4, 6 and 4 PFGE patterns were observed by *Xba*I, *Bln*I and *Spe*I digestion, respectively (Table 1). We named the dominant PFGE pattern as type 1 in each restriction endonuclease. A total of nine genetic subtypes were observed by a combination of these PFGE types (Table 1). The number of fragment differences from type 1 was less than four in each enzyme except the isolate 6-0 (Table 1). As these differences can be theoretically explained by less than two genetic events, these isolates may be derived from a common parent [12]. The isolate 6-0 might have different origin than the other isolates.

We observed continuous faecal shedding of *E. coli* O157:H7 for more than 2 months in one cow (no. 3). We also observed a shift of PFGE profile in the isolates from this cow. Especially after 6 November, changes in the genetic group of the isolates were observed every week. While we detected the *stx*2 gene, but not *stx*1 by PCR using the method of Pollard and colleagues [13] from the most of the isolates used in this study, neither gene was detected from the 11 isolates belonging to genetic subtypes III, IV, V and VI from cow no. 3 (Table 1). Karch and colleagues [14] observed a similar phenomena in human patients. That is, the shift of PFGE profile and the loss of *stx2* gene were observed among *E. coli* O157:H7 isolates from three of seven long-term human shedders. They suggested that the loss of *stx2* gene could be resulted from the genetic changes detectable by PFGE. It is not clear that the shift of PFGE pattern in the isolates from cow no. 3 was represented by a reinfection from the environment, a double infection, or a result of genetic changes and clonal replacement on the dominant genetic subtype within the cow. Since all but one isolate used in this study, however, were highly related, we assumed that a single strain that colonized cattle on this farm could have mutated slightly during either carriage by cattle, or in the farm environment.

The shift of PFGE pattern was also observed in two other cattle. The fact that the shift of genetic subtype was observed in three of the four cattle from which *E. coli* O157:H7 were isolated more than twice, including the examination of 20 September or 3 October, is important for investigators who wish to make inferences regarding genetic relatedness of *E. coli* O157:H7 isolates using PFGE patterns.

Faith and colleagues [7] observed up to three distinguishable, but closely related PFGE patterns among multiple isolates from the same cow. Keene and colleagues [11] also observed seven distinguishable PFGE patterns in three homology groups which differed by less than three bands among human and dairy herd *E. coli* O157:H7 isolates in a prolonged outbreak caused by raw milk. The authors suggested that outbreaks are not necessarily marked by a single pattern. Our results corroborate this opinion. Similarity, rather than exact matching, arguably gives a better understanding of the outbreak of *E. coli* O157:H7.

We thank Drs Dale Hancock, Thomas Besser, Margaret Davis, Jeffrey LeJeune and Daniel Rice (Washington State University) for helpful comments on preparing manuscript. Financial support was provided by the Ministry of Agriculture, Forestry and Fisheries of the Japanese Government.

REFERENCES

1. Armstrong GL, Hollingsworth J, Morris Jr JG. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiol Rev. 1996; **18**: 29–51.
2. Parry SM, Salmon RL, Willshaw GA, et al. Haemorrhagic colitis in child after visit to farm visitor centre. Lancet 1995; **346**: 572.
3. Rice DH, Hancock DD, Vetter RL, Besser TE. *Escherichia coli* O157 infection in a human linked to exposure to infected livestock. Vet Rec 1996; **138**: 311.
4. Böhm H, Karch H. DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulsed-field gel electrophoresis. J Clin Microbiol 1992; **30**: 2169–72.
5. Barrett TJ, Lior H, Green JH, et al. Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157:H7 by using pulsed-field gel electrophoresis and phage typing. J Clin Microbiol 1994; **32**: 3013–7.

6. Meng J, Zhao S, Zhao T, Doyle MP. Molecular characterization of *Escherichia coli* O157:H7 isolates by pulsed-field gel electrophoresis and plasmid DNA analysis. J Med Microbiol 1995; **42**: 258–63.
7. Faith NG, Shere JA, Brosch R, et al. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. Appl Environ Microbiol 1996; **62**: 1519–25.
8. Krause U, Thomson-Carter FM, Pennington TH. Molecular epidemiology of *Escherichia coli* O157:H7 by pulsed-field gel electrophoresis and comparison with that by bacteriophage typing. J Clin Microbiol 1996; **34**: 959–61.
9. Lee M, Kaspar CW, Brosch R, Shere J, Luchansky JB. Genomic analysis using pulsed-field gel electrophoresis of *Escherichia coli* O157:H7 isolated from dairy calves during the United States national daily heifer evaluation project (1991–1992). Vet Microbiol 1996; **48**: 223–30.
10. Izumiya H, Terajima J, Wada A, et al. Molecular typing of enterohemorrhagic *Escherichia coli* O157:H7 isolated in Japan by using pulsed-field gel electrophoresis. J Clin Microbiol 1997; **35**; 1675–80.
11. Keene WE, Hedberg K, Herriott DE, et al. A prolonged outbreak of *Escherichia coli* O157:H7 infections caused by commercially distributed raw milk. J Infect Dis 1997; **176**: 815–8.
12. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–9.
13. Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J Clin Microbiol 1990; **28**: 540–5.
14. Karch H, Russmann H, Schmidt H, Schwarzkopf A, Heesemann J. Long-term shedding and clonal turnover of enterohemorrhagic *Escherichia coli* O157 in diarrheal diseases. J Clin Microbiol 1995; **33**: 1602–5.