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Duration and magnitude of faecal shedding of Shiga toxin-producing *Escherichia coli* from naturally infected cattle

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SUMMARY

To clarify the epidemiological relationship between cattle and human infections of Shiga toxin-producing *Escherichia coli* (STEC), we studied the duration and magnitude of the excretion of STEC O157 and STEC O26 with rectal faeces from naturally infected cattle at a breeding farm in the Tohoku area of Japan, using microbiological methods. The prevalence of STEC O157 was 3·5% (11/324), whereas that of STEC O26 was 7·9% (14/178). Faecal shedding of STEC O157 persisted for <1 week to 10 weeks, whereas STEC O26 persisted from <1 week to <3 weeks. The magnitude of faecal shedding (per 10 g) ranged from 4 to $>110\,000$ c.f.u. for STEC O157 and from 3 to 2400 c.f.u. for STEC O26. All isolates of both STEC serotypes contained the *stx1* or *stx2* genes. Pulsed-field electrophoretic analysis of both STEC serotypes identified predominantly STEC O157 type III and STEC O26 type I in isolates, suggesting that a single STEC strain may be mutated in the intestinal tract of calves. These results indicate that STEC O157 is secreted for longer periods and in higher numbers than STEC O26 from healthy calves with natural infections, suggesting that STEC O157 may have more opportunities than STEC O26 to induce human disease.

INTRODUCTION

Certain Shiga toxin (Stx)-producing *Escherichia coli* (STEC) cause bloody diarrhoea in humans, leading to haemorrhagic colitis and haemolytic uraemic syndrome (HUS). These STEC are called enterohaemorrhagic *E. coli* (EHEC). Serious outbreaks of food poisoning associated with strains of STEC serotype O157:H7 have occurred in many countries. Only a few serogroups of STEC, including O26, O111 and O157, have caused the majority of outbreaks or sporadic cases of HUS [1–3]. Because STEC have often been isolated from the faeces of beef and dairy cattle [4–7], and two cases of food poisoning have been reported in Japan after the consumption of beef liver contaminated with STEC [8], cattle are thought to be one of the potential reservoirs of STEC infections.

Our previous study showed that the detection frequency of *stx* genes (*stx1*, *stx2*, and *stx1+2*) was quite similar in STEC isolated from faecal specimens from calves at a breeding farm and from cattle at a slaughterhouse in the Tohoku area of Japan [9]. However, there have been no data to verify the correlation between high infection rates in calves and human infections with STEC. Although STEC O157 has been more frequently isolated from bovine products and faecal samples than STEC O26, little

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Table 1. *Prevalence of STEC in healthy cattle by season in 2001*

STEC type	Number of STEC-positive cattle/number of cattle tested			
	Winter (1 Feb.–19 Feb.)	Spring (1 May–13 June)	Summer (3 July–2 Aug.)	Total (%)
O157	2/106 (1·9%)	—/166	9/52 (17·3%)	11/324 (3·4%)
O26	3/106 (2·8%)	9/52 (17·3%)	2/20 (10%)	14/178 (7·9%)

is known about the faecal excretion of STEC O157 and/or STEC O26 which are taken from naturally infected cattle. Therefore, we studied the duration and magnitude of shedding of both STEC serotypes in the rectal faeces of naturally infected calves at a breeding farm in the Tohoku area, a region in the northern area of Japan.

MATERIALS AND METHODS

Sampling

Rectal specimens were collected from a total of 324 rectal stool grab samples from naturally infected calves at a breeding farm in the Tohoku area of Japan. The faecal specimens were collected in three seasons during 2001: winter (1–19 February); spring (1 May–13 June) and summer (3 July–2 August) (see Table 1). A total of 324 rectal specimens, from 233 Holstein (H), 28 Japanese Black (B), and 63 H × B (F1) cattle aged 1–10 months were tested for the presence of STEC O157; and 178 rectal specimens, from 123 H, 24 B, and 31 F1 cattle aged 1–10 months were tested for STEC O26. Rectal samples were collected once a week for 14 weeks to test for STEC O157, and for 6 weeks to test for STEC O26. Each season, the rectal samples were collected from different calves at different cattle sheds in a farm.

Experimental animals

This study was performed with calves from a breeding farm in the Tohoku area. Naturally infected calves aged 1–10 months of different breeds from five herds (herds A–E), were selected randomly; 30–50 rectal samples were tested each time for initial screening of STEC O157 and STEC O26. For further investigation, the positive calves were examined again and this was designated as week 0 for investigation of the duration of faecal shedding. All rectal samples were taken independently in different seasons, age and breed of cattle are presented in Table 2.

Faecal sample

Rectal samples were obtained aseptically from calves by rectal palpation. Briefly, the faeces were transported to the laboratory by ice box (4–8 °C) and taken to the laboratory for immediate processing (usually within 24 h).

Isolation of STEC (O157 and O26)

Enrichment culture

Faecal specimens (1 g) were cultured (37 °C, 18 h) in mEC broth (9 ml) with novobiocin (25 μg/ml; Kyokuto-Kagaku, Tokyo, Japan) for selective enrichment as previously described [9–12]. After enrichment, 1 ml of the culture was inoculated into 9 ml of tryptosoy enrichment medium (Nissui-Seiyaku, Tokyo, Japan) and cultured at 37 °C for 18 h.

Immunomagnetic separation (IMS) followed by culture to sorbitol MacConkey containing cefixime tellurite (CT-SMAC) agar and rhamnose MacConkey containing cefixime tellurite (CT-RMAC) agar for detecting E. coli *O157 and O26*

The IMS technique involves coating magnetic beads with antibody against *E. coli* (principally O157) [13]. Inclusion of an IMS step in the isolation procedure enhanced this sensitivity by at least 100-fold in detecting the organism than by direct culture [11, 14–20]. Enrichment culture (1 ml) was then added to 20 μl of magnetic beads coated with antibody against *E. coli* O157 (Dynabeads anti-*E. coli* O157; Dynal, Oslo, Norway) and *E. coli* O26 (Denka Seiken, Tokyo, Japan) in a 1·5-ml microcentrifuge tube. The beads were suspended evenly in the broth culture by vortex mixing and were then placed in a rotating mixer so that they were mixed by inversion every 2–3 s for 30 min at ambient temperature. Tubes were placed in a magnetic particle concentrator (MPC-10; Dynal), and the magnets were put in position and left for 5 min. The culture supernatant was removed by aspiration with a pipette, the magnetic slide was removed from the rack, the beads were washed by resuspension in 1 ml of sterile physiological saline, and the magnetic slide was replaced for 2 min. The beads were washed twice in the same way, then the supernatant was removed and the beads were

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Table 2. *Duration and fecal shedding of STEC O157 cells in healthy cattle*

No.	Breed of cattle*	Age of cattle (months)	Sampling season†	Number of STEC O157 cells (c.f.u./10 g faeces) per week and (*stx* type)													
				0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk	8 wk	9 wk	10 wk	11 wk	12 wk	13 wk	14 wk	15 wk
1	H	4	W	>110 000 (2)	>110 000 (2)	>110 000 (2)	2400 (2)	—	53 (2)	—	—	—	—	NT	NT	NT	NT	NT	NT
2	H	6	W	2400 (1/2)	380 (2)	11 000 (1/2)	—	—	—	4 (2)	4 (1/2)	—	—	—	—	NT	NT	NT	NT
3	B	5	Sm	44 (2)	—	—	23 (2)	11 (2)	23 (2)	9 (2)	—	—	—	—	NT	NT	NT	NT	NT
4	F1	5	Sm	9 (2)	—	—	23 (2)	23 (2)	2400 (2)	2400 (2)	17 (2)	14 (2)	15 (2)	—	—	—	—	NT	NT
5	B	5	Sm	14 (2)	150 (2)	1100 (2)	>110 000 (2)	1100 (2)	2400 (2)	1700 (2)	4 (2)	14 (2)	15 (2)	—	—	—	—	NT	NT
6	F1	3	Sm	11 000 (2)	240 (2)	2400 (2)	29 (2)	>110 000 (2)	24 000 (2)	46 000 (2)	13 (2)	11 (2)	7 (2)	38 (2)	—	—	—	—	NT
7	H	4	Sm	2400 (2)	23 (2)	—	9 (2)	—	4 (2)	—	—	—	—	NT	NT	NT	NT	NT	NT
8	H	5	Sm	460 (2)	4 (2)	—	—	4 (2)	—	—	—	—	NT	NT	NT	NT	NT	NT	NT
9	F1	5	Sm	43 (2)	93 (2)	—	9 (2)	20 (2)	—	290 (2)	29 (2)	11 (2)	4 (2)	—	—	—	—	NT	NT
10	H	2	Sm	160 (2)	—	—	—	—	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
11	B	10	Sm	4 (2)	NT	NT	1700 (2)	—	4 (2)	—	15 (2)	—	—	—	—	NT	NT	NT	NT

* H, Holstein; B, Japanese Black; F1, H × B.
† W, winter; Sm, summer.
NT, Not tested for sample that continued negative until weeks 3 and 4, confirmation of consecutive negative cultures.

resuspended in 100 μl of sterile physiological saline. The concentrated magnetic beads were plated onto CT-SMAC (Dynal) agar plates for O157 or CT-RMAC agar plates for O26 and incubated at 37 °C for 24 h. CT-RMAC agar contained 20·0 g peptone (Difco, Detroit, MI, USA), 1·5 g bile salts no. 3 (Difco), 11·1 g rhamnose monohydrate (Kanto Chemical, Tokyo, Japan), 5·0 g NaCl (Wako, Osaka, Japan), 0·03 g Neutral Red (Wako), 0·001 g Crystal Violet (Kanto Chemical), and 15·0 g Bacto agar (Difco) added to 1 l Milli-Q water. After the pH was adjusted to 7·2 with HCl (Wako), the medium was autoclaved at 121 °C for 15 min and cooled to 50 °C. Cefixime tellurite (Oxoid, Hampshire, UK) was added aseptically to the medium. The mixture was then poured into sterile Petri dishes [21]. After overnight incubation at 37 °C, the five positive colonies grown on CT-SMAC and CT-RMAC agar were picked up and inoculated onto HI agar and incubated at 37 °C for 24 h. Five apparently non-sorbitol-fermenting colonies were examined for agglutination test with antisera directed against *E. coli* serogroups O157 or O26. After the *E. coli* serogroups were determined, the presence of *stx* genes was confirmed by PCR. The positive numbers of STEC O157 and O26 cells in faeces were determined using the most probable number (MPN) method.

MPN method for estimating the concentration of STEC in positives samples

Numbers of STEC O157 and O26 cells in faeces were estimated using the MPN method. Positive STEC O157 and O26 specimens were divided into three categories: 1 g, 0·1 g and 0·01 g. A sample for each category was inoculated into each of three tubes containing of 9 ml of novobiocin (N)-mEC medium. The MPN was obtained by determining the sum of the positive and negative tubes by a previously described protocol [22, 23]. *Stx*-positive specimens were inoculated into N-mEC broth at 37 °C for 18 h. IMS of pre-enriched culture samples was performed with magnetic beads as described above. The numbers of STEC O157 and O26 cells in faeces were determined using standard MPN tables. STEC O157 and O26 were isolated by the MPN method during the faecal shedding of both.

PCR

The presence of *stx1* and *stx2* was examined by PCR as described previously [9, 13, 19, 24, 25]. One ml of

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Table 3. *Duration and faecal shedding of STEC O26 cells in healthy cattle*

No.	Breed of cattle*	Age of cattle (months)	Sampling season†	Number of STEC O26 cells (c.f.u./10 g faeces) per week and (*stx* type)						
				0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk
1	F1	1	W	3 (1)	—	—	—	—	NT	NT
2	B	2	W	21 (1)	1100 (1)	—	—	—	—	NT
3	B	2	W	29 (1, 2)	—	—	—	—	NT	NT
4	H	6	Sp	4 (1)	—	—	—	—	NT	NT
5	H	6	Sp	4 (1)	9 (1)	—	—	—	—	NT
6	H	5	Sp	2400 (1)	36 (1)	—	—	—	—	NT
7	H	5	Sp	4 (1)	—	—	—	—	NT	NT
8	H	5	Sp	4 (1)	4 (1)	—	—	—	—	NT
9	H	5	Sp	9 (1)	43 (1)	23 (1)	—	—	—	—
10	H	5	Sp	29 (1)	210 (1)	21 (1)	—	—	—	—
11	H	5	Sp	4 (1)	—	—	—	—	NT	NT
12	H	5	Sp	3 (1)	—	—	—	—	NT	NT
13	H	1	Sm	17 (1)	—	—	—	—	NT	NT
14	H	1	Sm	15 (1)	15 (1)	19 (1)	—	—	—	—

* H, Holstein; B, Japanese Black; F1, H × B.
† W, winter; Sp, spring; Sm, summer.
NT, Not tested for samples that continued negative until weeks 3 and 4, confirmation of consecutive negative cultures.

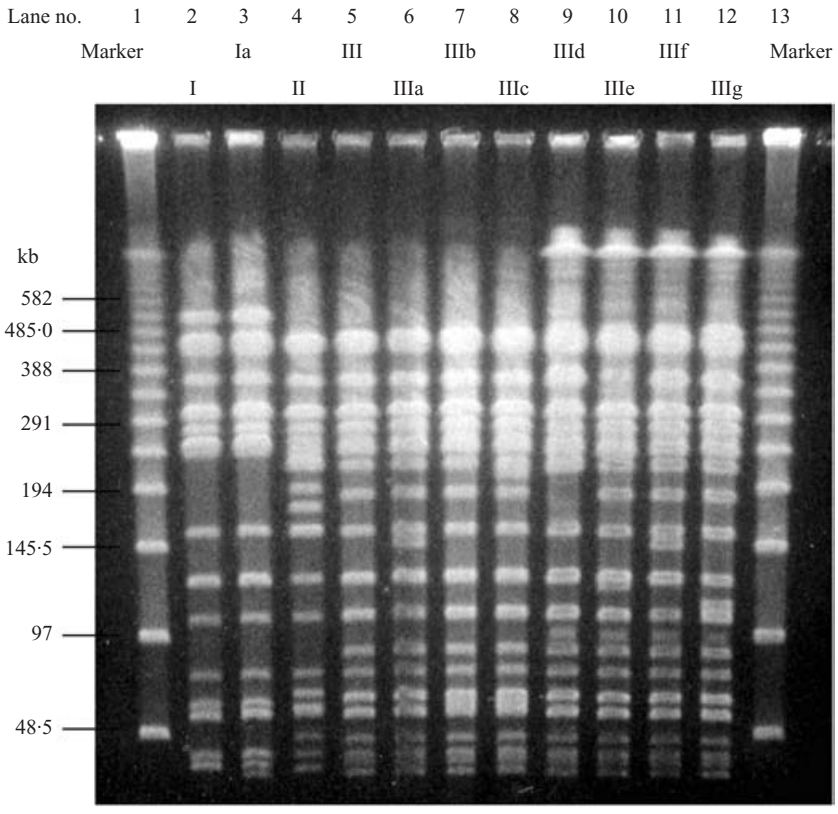

Fig. 1. Representative PFGE patterns of *Xba*-digested genomic DNA of STEC O157. Each PFGE pattern indicates a clonal subgroup. Lane 1, lambda ladder marker; lane 2, I pattern (calf no. 1); lane 3, Ia pattern (calf no. 1); lane 4, II pattern (calf no. 2); lane 5, III pattern (calf nos. 3–11); lane 6, IIIa pattern (calf no. 3); lane 7, IIIb pattern (calf no. 5); lane 8, IIIc pattern (calf no. 5); lane 9, IIId pattern (calf no. 6); lane 10, IIIe pattern (calf nos. 4, 7, 9); lane 11, IIIf pattern (calf no. 11); lane 12, IIIg pattern (calf no. 11); lane 13, lambda ladder marker.

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Table 4. stx *gene type and PFGE pattern of STEC O157 isolates from a cattle*

Cattle no.	*stx* gene type/PFGE pattern of isolates*														Cattle herd
	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk	8 wk	9 wk	10 wk	11 wk	12 wk	13 wk	14 wk	15 wk	
1	2/I	2/I	2/I, Ia†	2/I	—‡	2/I	—	—	—	—	—	NT§	NT	NT	NT	NT	A
2	1&2‖/II	1&2/II	1&2/II	—	—	—	1&2/II	1&2/II	—	—	—	—	NT	NT	NT	NT	B
3	2/III	—	—	2/III	2/III	2/III	2/IIIa	—	—	—	—	NT	NT	NT	NT	NT	
4	2/III	—	—	2/III	2/III	2/III	2/III	2/III	2/III	2/III, IIIe	—	—	—	—	NT	NT	
5	2/III	2/IIIb	2/IIIc	2/III, IIIb	2/IIIc	2/IIIb	2/IIIb	2/IIIb	2/III	2/III, IIIb	—	—	—	—	NT	NT	
6	2/III	2/III	2/III	2/III	2/III	2/III	2/III	2/III, IIId	2/III	—	2/IIId	—	—	—	—	NT	C
7	2/III	2/III	—	2/IIIe	—	2/III	—	—	—	—	NT	NT	NT	NT	NT	NT	
8	2/III	2/III	—	—	2/III	—	—	—	—	NT	NT	NT	NT	NT	NT	NT	
9	2/III	2/III	—	2/III	2/III	—	2/IIIe	2/IIIe	2/IIIe	2/IIIe	—	—	—	—	NT	NT	
10	2/III	—	—	—	—	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	D
11	2/IIIf	NT	NT	2/IIIf	—	2/III, IIIg	—	2/III	—	—	—	—	NT	NT	NT	NT	E

* Two isolates from a calf were used for determination of *stx* type and PFGE pattern.
† One showed pattern I by PFGE analysis whereas the second showed pattern Ia.
‡ —, STEC O157 was not detected from a calf.
§ NT, detection of STEC O157 was not tried.
‖ Both of *stx1* and *stx2* genes were detected in the same STEC O157 isolate.

culture was centrifuged at 10 000 ***g*** for 10 min. The pellet was resuspended in 1 ml of sterile distilled water and then boiled for 10 min. After boiling, the suspension was centrifuged again, and the supernatant used for the PCR reaction. To detect *stx* genes in cultures of faecal specimens, we used PCR primer pairs for *stx1* (EVT1 and EVT2) and *stx2* (EVS1 and EVS2) (Takara Shuzou, Tokyo, Japan). PCR was run for 35 cycles: denaturation at 94 °C for 60 s; annealing at 55 °C, 60 s; and extension at 72 °C, 60 s. The amplification products (10 μl) were analysed by electrophoresis in a 1% agarose gel (Seakem, Rockland, ME, USA) for 1 h and were stained with ethidium bromide.

Pulsed-field gel electrophoresis (PFGE)

The confirmed *E. coli* O157 and O26 isolates further were subtyped by PFGE separation of *Xba*I-digested genomic DNA. Genomic DNA was prepared and embedded in agarose, as described previously [26], and digested with *Xba*I (Boehringer–Mannheim, Mannheim, Germany). PFGE was performed with CHEF DRIII (Bio-Rad, Richmond, CA, USA) equipment and 1·2% agarose gels at 14 °C for 42 h, at a constant voltage of 200 V. Pulse times were ramped for 12·6 s at the beginning and 40 s at the end. Chromosomal DNA from *E. coli*, digested with *Xba*I, was used as a molecular marker. Restriction fragment patterns of genomic DNA were determined using the GelScan System and analysed with the RFLPScan software (Scanalytics, Fairfax, VA, USA). PFGE banding patterns were examined visually, and each unique banding pattern was assigned a PFGE pattern number [27]. The categories of genetic and epidemiological relatedness of PFGE patterns are based on the criteria described previously [28]. A shift in pattern suggests a genetic change and clonal replacement within cattle is referred to as 'clonal turnover' [29].

RESULTS AND DISCUSSION

Prevalence of STEC O157 and O26 in naturally infected cattle

From the initial samples, we isolated two types of STEC; both STEC O157 and O26 were not isolated from the same rectal sample. STEC O157 were recovered from 11 (3·5%) of 324 cattle rectal samples, whereas STEC O26 were recovered from 14 (7·9%)

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of 178 cattle rectal samples. STEC O157 were shed by calves aged 2–10 months, whereas STEC O26 were excreted by calves aged 1–7 months. The highest prevalence of faecal shedding was found in calves aged 5–6 months for STEC O157 and in calves aged 1–2 months for STEC O26. Although the difference cannot be accounted for at the present time, this might be caused by the condition of the ruminants and the intestinal tract of each calf. There was considerable variability in the faecal excretion of STEC O157 for young and adult cattle naturally and experimentally infected with STEC O157 [1, 11, 30, 31].

The results were summarized on the basis of the different cattle breeds (data not shown). They show that the greatest excretion of STEC O157 was found in Japanese Black calves (10·7%), whereas the greatest excretion of STEC O26 was in Holstein calves (8·9%). With regard to excretion rates of both STEC by seasonal variation, STEC O157 were isolated from rectal faeces of 17·3% of cattle in summer, whereas STEC O26 were isolated from 17·3% of cattle in spring and from 10% of cattle in summer. Shedding peaked during spring and summer, but extremely low rates of shedding were observed in winter. These peaks are consistent with the high incidence of food poisoning by STEC in summer and spring. The seasonal variation in the shedding of STEC O157 and O26 has also been reported previously [10, 11, 17, 32–34]. However, there have been no reports of the faecal excretion of STEC O26 by naturally infected calves.

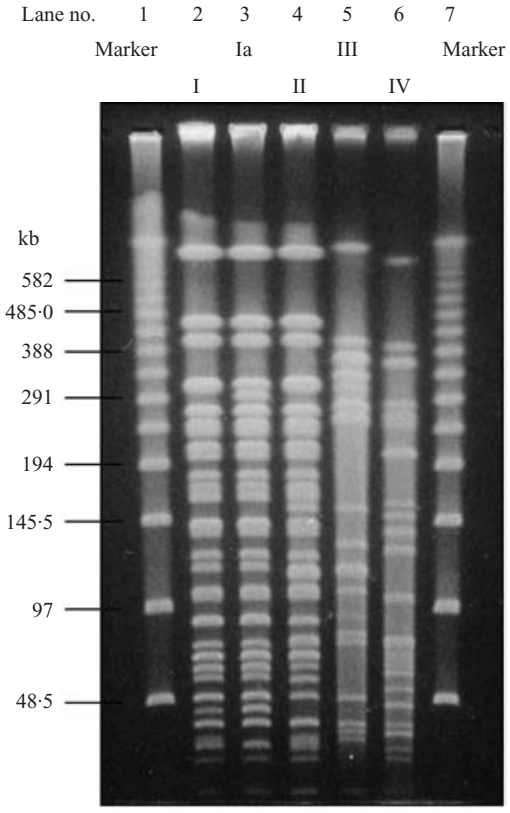

Fig. 2. Representative PFGE patterns of *Xba*-digested genomic DNA of STEC O26. Each PFGE pattern indicates a clonal subgroup. Lane 1, lambda ladder marker; lane 2, I pattern (calf nos. 5–12); lane 3, Ia pattern (calf no. 10); lane 4, II pattern (calf no. 13); lane 5, III pattern (calf no. 4); lane 6, IV pattern (calf no. 2); lane 7, lambda ladder marker.

Duration and magnitude of faecal shedding of STEC O157 and O26 by cattle

Results were defined as negative when no STEC was isolated from 10 g of examined faeces. To our knowledge, the negative results designate clearance of *E. coli*. Faecal excretion of STEC O26 ceased after 1–3 weeks, as shown in Table 3, whereas faecal excretion of STEC O157 mainly ceased after 1–10 weeks (Table 2). Not tested (NT) indicates that no STEC was isolated in the 4 weeks of the investigation respectively. Faecal shedding of STEC O157 from calves was examined every week for 14 weeks, and duration of faecal STEC O157 shedding varied from 1 week (calf no. 10) to 10 weeks (calf no. 6) (Table 2). Calf nos. 8 and 10 did not excrete STEC O157 in faeces for <4 weeks. Calf nos. 1, 2, 3, 7 and 11, shed STEC O157 for 5–7 weeks, whereas calf nos. 4, 5, 6 and 9 did so for 9–10 weeks. Among the calves examined in this study, a widely varying duration of faecal shedding of STEC occurred. From these cattle were grouped as short-term ($\leqslant 1$ week) and long-term ($\geqslant 10$ weeks).

The level of STEC O157 in faeces ranged from 4 to $>110\,000$ c.f.u./10 g. This range was classified into three groups based on the shedding patterns; e.g. 1–100 c.f.u./10 g for calf no. 3, 1–1000 c.f.u./10 g for calf nos. 8, 9 and 10, and from 1 to $>110\,000$ c.f.u./10 g for calf nos. 1, 2, 4, 5, 6, 7 and 11.

The duration and magnitude of STEC O26 shedding in faeces are presented in Table 3. Faecal shedding of STEC O26 was examined once a week for 6 weeks. Faecal STEC O26 were shed by calf nos. 9 and 10 for 2 weeks, and for <1 week by calf nos. 1, 3, 4, 7, 11, 12 and 13. The amount of STEC O26 shed in faeces was 3–2400 c.f.u./10 g. This range was divided into three groups: 1–10 c.f.u./10 g for calf nos. 1, 4, 5, 7, 8, 11 and 12; 1–100 c.f.u./10 g for calf nos. 3, 9, 13 and 14; and from 1 to >1000 c.f.u./10 g for calf nos. 2, 6 and 10. The present findings demonstrate that

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Table 5. stx *gene type and PFGE pattern of STEC O26 isolates from a cattle*

Cattle no.	*stx* gene type/PFGE pattern of isolates*									Cattle herd
	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk	8 wk	
1	1/UT†	—‡	—	—	NT§	NT	NT	NT	NT	A
2	1/IV	1/UT	—	—	—	—	NT	NT	NT	B
3	1, 2‖/UT	—	—	—	—	NT	NT	NT	NT	C
4	1/III	—	—	—	—	NT	NT	NT	NT	D
5	1/I	1/I	—	—	—	—	NT	NT	NT	D
6	1/I	1/I	—	—	—	—	NT	NT	NT	E
7	1/I	—	—	—	—	NT	NT	NT	NT	E
8	1/I	1/I	—	—	—	—	NT	NT	NT	E
9	1/I	1/I	1/I	—	—	—	—	NT	NT	E
10	1/Ia	1/I	1/I	—	—	—	—	NT	NT	E
11	1/I	—	—	—	—	NT	NT	NT	NT	E
12	1/I	—	—	—	—	NT	NT	NT	NT	E
13	1/II	—	—	—	—	—	—	NT	NT	F
14	1/UT	1/UT	1/UT	—	—	—	—	NT	NT	G

* Two isolates from a calf were used for determination of *stx* gene type and PFGE pattern.
† UT, untypable because of appearance of smeared bands.
‡ —, STEC O157 was not detected.
§ NT, detection of STEC O157 was not tried.
‖ Both *stx1* and *stx2* genes were detected in the same STEC O26 isolate.

the prevalence of both STEC O157 and O26 are statistically different to those of STEC O26 ($P<0{\cdot}05$). Moreover, the duration and magnitude of shedding differ for each serotype. STEC O157 were shed for a longer period and in higher numbers in faeces than STEC O26, indicating that there is a higher risk of possible human infection by STEC O157 than by STEC O26. STEC O26 were excreted in faeces for up to 2 weeks (Table 3), indicating transient infections of STEC O26. On the other hand, STEC O157 were excreted in faeces for up to 10 weeks (Table 2), indicating persistent infections of STEC O157. These results are consistent with the previous findings that calves experimentally infected with STEC O157 shed these bacteria for a longer period than calves infected with STEC O26 [18, 33, 35–37]. As yet, it is unclear why the period of excretion of STEC O26 is so short. It worth noting that higher rates of infection due to STEC O26, as observed in this study, could also be a risk factor, although its excretion in lower numbers in faeces could make it less efficient for transmission compared to STEC O157.

The reason why the difference in the magnitude of STEC O157 and STEC O26 shedding is unclear, although some physiological features of the bovine intestinal environment may influence the growth and proliferation of both STEC serotypes. These findings should be useful in assessing the persistent excretion of STEC O157 and O26 by naturally infected individual calves.

PCR and PFGE

Stx genes were identified in STEC isolates by PCR. Of the 11 isolates (two strains/animal) of STEC O157 presented in Table 2, both *stx1* and *stx2* genes were detected in an isolate from calf no. 2, whereas only the *stx2* gene was detected in the other 10 isolates. On the other hand, the *stx1* gene was detected in 14 isolates of STEC O26, whereas both the *stx1* and *stx2* genes were detected in one isolate from calf no. 3 (Table 3).

The genomic DNA patterns of STEC isolates were analysed by PFGE. As shown in Figure 1, the *Xba*I PFGE patterns of STEC O157 isolates differed. The genomic profiles of the STEC O157 isolates were classified into three types (types I, II and III), according to the differences in their patterns. These types were further classified into subtypes: Ia exhibited a slight shift in pattern from type I; and subtypes IIIa–IIIg each showed unique distinguishable PFGE banding pattern when compared with the lambda ladder marker.

Genomic DNA analysis by PFGE of STEC O157 isolates from calf no. 1 identified the type I pattern at weeks 0, 1, 3 and 5, whereas the type Ia pattern

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was found in week 2. Analysis of the DNA of STEC O157 from calf no. 2 identified the type II pattern at weeks 3, 4, 5, 9 and 10. DNA analysis of STEC O157 from calf no. 3 identified the type III pattern at weeks 0 and 3. The same type III pattern appeared predominantly in the STEC O157 isolates, whereas the type I pattern predominated in the STEC O26 isolates, indicating that the same bacteria were carried by many of the cattle during the period of excretion. DNA patterns of STEC O157 from calf nos. 5, 6 and calf nos. 4, 7, 9 corresponded to subtypes IIIb, IIIc, IIId and IIIe, as presented in Table 4.

The genomic DNA profiles of STEC O26 were classified into four groups by PFGE analysis, as shown in Figure 2. The diversity of each pattern was further classified by the criteria used for STEC O157. The type I pattern was observed in the DNA of STEC O26 from calf nos. 5–12, as shown in Table 5. Subtype Ia, with a pattern slightly shifted from that of type I, and types II, III and IV DNAs were identified in STEC O26 from calf nos. 10, 13, 14 and 3 respectively. No subtypes with types II, III and IV patterns were observed (Table 5). Genomic DNA analysis of STEC by PFGE is a valuable tool with which to evaluate whether the same STEC strain persistently infects an individual calf or calves in a herd. The excretion of STEC O26 was transient. The PFGE patterns of the STEC O26 isolates were mainly type I, suggesting that the same STEC O26 strain infected these calves. On the other hand, the excretion of STEC O157 ceased in some calves although type III strains of STEC O157 were found to predominantly and persistently infect most calves tested. STEC O157 subtypes IIIa–IIIg were also identified. Although type I strains of STEC O26 were predominant, STEC O26 subtypes Ia and III were also found. These data may indicate that the genomic DNA of both STEC serotypes is partly mutated in the intestinal tracts of calves, as suggested by Akiba et al. [38]. Because we picked only two single colonies in the confirmed STEC O157 and STEC O26 isolates, we therefore studied only two isolates of STEC by PFGE analysis. However, to confirm that the changes in the PFGE pattern observed in this study arise as the result of mutation in the intestinal tract of calves, further study is required to analyse the PFGE patterns of more isolates.

The present results demonstrate that STEC O157 infections of calves at a breeding farm in the Tohoku area of Japan are persistent, whereas STEC O26 infections of these calves were transient. Therefore, STEC O157 may more frequently infect bovine products than STEC O26, suggesting that STEC O157 is the pathogen more likely to induce food poisoning.

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