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High numbers of Shiga toxin-producing *Escherichia coli* found in bovine faeces collected at slaughter in Japan

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Abstract

The prevalence and concentration of Shiga toxin-producing *Escherichia coli* (STEC) in cattle faeces ($n = 605$) at the time of slaughter was studied in Shimane Prefecture, Japan on a monthly basis between April 2000 and March 2001. Screening with *stx*-PCR determined a prevalence of 37.5%. After analysis of spread faeces and enriched samples on cefixime, tellurite and sorbitol-MacConkey agar using HCl treatment, 114 STEC strains were singly or concomitantly isolated from 97 cattle (15.9%). Of the 605 cattle, 31 (5.1%) harbored O26:H11, O111:H-, O121:H19 or O157:H7, which had the *stx1* and/or *stx2* and *eae* and *hlyA* genes, and 7 (23%) of these 31 cattle were high level carriers that contained these typical STEC at concentrations of 10^5–10^8 CFU/g of faeces. The predominant serotype was O26:H11 (20 strains) and the second most frequent was O157:H7 (9 strains). Of the 605 cattle, 68 (11.2%) harboured 36 other serotypes and 6 (5.9%) of the 67 cattle were high level carriers. As a comparison between the prevalence of STEC and the faecal pH, it was demonstrated that STEC can be isolated from cattle with a wide range of faecal pH values. The presence of a high-carriage animal at the abattoir increases the potential risk of meat contamination during the slaughtering process, regardless of faecal pH.

Keywords: Shiga toxin-producing *Escherichia coli*; Cattle; EHEC 0157; Faeces; EHEC 026; EHEC 0111

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) strains of different serotypes have been increasingly isolated from humans with disease and from healthy domestic animals [1,2]. Many of these isolates were typical STEC strains belonging to serotypes O26:H11, O103:H2, O111:H-, O121:H19 and O157:H7, which in humans can cause sever disease such as hemorrhagic colitis and hemolytic-uraemic syndrome. Cattle and other ruminants have been established as major natural reservoirs for STEC O157 [3] and play a significant role in the epidemiology of human infections [4]. It has been estimated that the prevalence rate of O157 in slaughtered cattle is 6.5% in Japan [5], although recently the prevalence of STEC O26, O103, O111 and O157 of 3.9% was reported for breeding cows on dairy farms in central Japan [6]. In our previous study of 151 breeding cattle on 8 farms in western Japan, the prevalence rates of O26:H11, O111:H- and O157:H7 were 19.2%, 7.3% and 20.5%, respectively [7]. Surveys performed in European countries, in North America, in South America, and in Asia showed a much lower, but also highly fluctuating prevalence of STEC on farms and at slaughter ranging from 0.5% up to 10% of positive animals [2]. Events related to slaughtered cattle significantly impact public health, although differences in the STEC

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prevalence rates in previous studies could be due to different seasonal and/or detection methods. Healthy cattle can be transient reservoirs of food-borne pathogens and many human infections result from ingestion of contaminated bovine food products [2,8–13]. Therefore, data on the prevalence and concentration of STEC in animals at slaughter are critical for the design of risk-based hazard analysis for critical control point programs in order to control pathogens.

Regarding risk assessment of infections in humans with STEC, the concentration of STEC shed from individual cattle entering the slaughter process is as important as the prevalence of STEC carrying by these cattle. The concentration at which STEC O157:H7 is shed in faeces varied from 10^2 to 10^5 CFU/g in a North American study with calves [14]. Faecal carriage of O157:H7 in cattle was transient [15] and significantly greater in calves than in previous farm surveys [14,16,17]. In experimentally induced infections in calves and adult cattle, calves shed O157:H7 in greater numbers and for a longer duration than did adult animals [18,19]. A recent study of O157:H7 in slaughtered cattle from the United Kingdom showed a range from 10^2 to 10^6 CFU/g in 7.5% of examined cattle [20]. However, little information is available on the concentration of STEC other than O157:H7 in adult cattle faeces at slaughter.

While various approaches have been used to develop isolation and detection procedures for STEC O157 [8,10], effective methods for isolation and detection of serotypes of STEC other than O157 have not been widely reported. Our group reported an effective, rapid, and simple method for isolation and counting of STEC O26, O111, and O157 from faeces and food samples [7,21]. We used culture on cefixime, tellurite and sorbitol-MacConkey agar [22] after HCl treatment of samples [7,21]. This new method is based on the acid resistance of *E. coli* and the tellurite resistance of *eae*-positive STEC strains.

Dissemination of acid-resistant generic *E. coli* organisms and/or O157:H7 from hay- and grain-fed cattle experimentally inoculated with these organisms has been compared [23,24]. Cattle fed grain diets have acid-resistant generic *E. coli* organisms in their faeces and it was suggested that feeding cattle hay diets would reduce the risk of food-borne O157:H7 infections for humans [23]. However, cattle fed hay diets shed O157:H7 longer than cattle fed grain diets, and bacteria from both were equally acid resistant [24]. This suggested that feeding cattle hay may increase the risk of human infection from O157:H7. However, the relationship between faecal pH and colonization of STEC in the colon is poorly understood in the case of slaughtered adult cattle.

The primary objective of this present study was to determine the prevalence and concentration of STEC in faeces of cattle slaughtered for beef consumption. The second objective was to determine the relationship between faecal pH and the presence of STEC in the colon of slaughtered adult cattle in order to search for effective methods to control STEC infections in cattle.

2. Materials and methods

2.1. Collection and storage of faecal samples

Between April 2000 and March 2001, 605 faecal specimens from 479 beef cattle (Japanese black cattle), 97 dairy cull cows (Holstein) and 29 F1 cattle (hybrid between Japanese black cattle and Holstein) were collected at a slaughterhouse in Shimane Prefecture, Japan. Samples (0.1 g) collected on cotton swabs from the faeces raked out from the rectum of the animals immediately after slaughter were kept in 0.9 ml of 0.85% NaCl solution and thereafter this 10-fold suspension of faeces was prepared. The specimens were kept at 4 °C during transportation and examined for the presence of STEC and *E. coli* within 4 h after sampling. The faecal pH was measured using a pH meter (Kouwa, Nagoya, Japan) at the time of slaughter.

2.2. Isolation of STEC by direct plating after HCl treatment

To count viable STEC and *E. coli* in faeces treated with HCl [7,21,25] (Fig. 1), a portion (0.3 ml) of these 10-fold diluted faecal samples was transferred to HCl solution (0.3 ml, 0.125 N HCl with 0.5% NaCl), mixed well and held for 30 s. Each 0.2 ml portion, which included 0.01 g of faeces, of the resulting HCl treated samples was then spread onto cefixime (0.05 mg/l), tellurite (2.5 mg/l), and sorbitol-MacConkey agar (CT-SMAC) [22] and Chromocult coliform (CC) agar (Merck) plates, respectively. Next, these 0.02 ml portions, which included 0.001 g of faeces, of the resulting HCl treated samples were quickly put into 1 ml of 0.067 M phosphate buffer saline (PBS, pH 7.2) for preparation of a 10^3-fold dilution of faeces and for neutralization of the HCl treatments and were spread onto CT-SMAC and CC agar plates, respectively. Portions (0.01 ml) of 10^3- and 10^5-fold dilutions were placed onto CT-SMAC and CC agar plates, respectively, thoroughly dried and then incubated at 37 °C for 18 h. The numbers of *E. coli* and STEC were calculated as colony-forming units (CFU). The blue colonies grown on CC agar were counted as *E. coli* when measuring CFU of this organism in the faeces. The presumptive O157:H7 colonies (non-sorbitol-fermenting colonies on CT-SMAC and the red colonies on CC agar) and the presumptive other STEC (sorbitol-fermenting colonies on CT-SMAC and the blue colonies on CC agar) were counted. Then, five colonies from each plate were further confirmed by

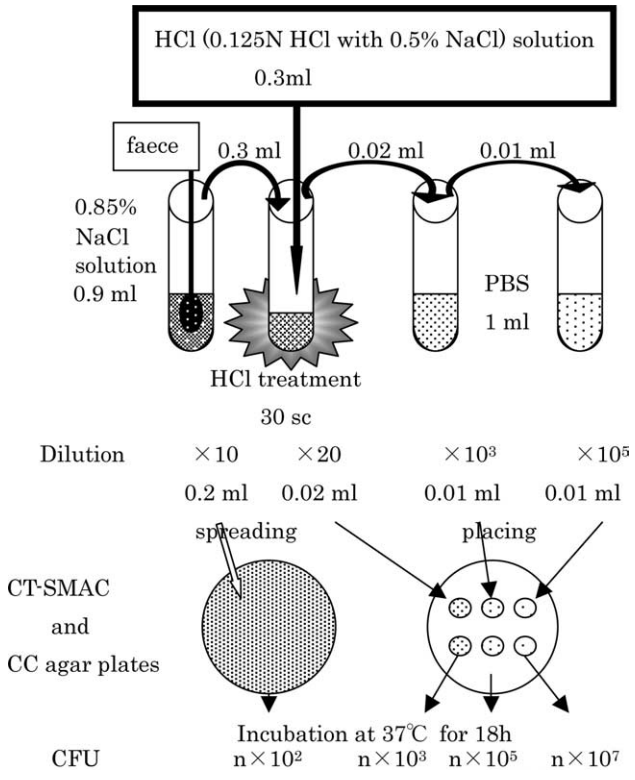

Fig. 1. CFU enumeration method after HCl treatment.

biochemical, serological and PCR methods and CFU of STEC in the faeces were finally counted.

2.3. Isolation of STEC by enrichment

For enrichment, the remaining portions of a 10-fold dilution of faeces were put into 10 ml of Trypticase soy broth (TSB, BBL) and incubated for 6 h at 42 °C. Iimmunomagnetic separation (IMS) was done using 1 ml of enrichment, according to instructions from the manufacturer. A mixture of magnetic beads (Dynabeads, Dynal) coated with an antibody against *E. coli* O26, O111 or O157 (Denka-Seiken, Niigata, Japan) was prepared according to instructions from the manufacturer. Each portion (0.02 ml) of the enrichment cultures and IMS-treated cultures, because IMS works well even with large numbers of non-target bacteria, was transferred to HCl solution (0.02 ml), mixed well, held for 30 s, and then 0.02 ml portions of the resulting HCl treated samples were spread onto CT-SMAC and CC agar plates. The agar plates were incubated at 37 °C for 18 h. The presence of STEC on the agar plates was screened by colony sweep assay using multiplex PCR to detect *stx* genes [26]. From the PCR-positive plates, 50 colonies from each plate were cultured on CC agar plates at 37 °C for 18 h. Samples pooled from five strains were tested for carriage of *stx* genes using multiplex PCR and then individual strains were tested again. The PCR-positive strains were further confirmed biochemically.

Table 1
The primers used in this study

Gene	Primer pair	References
eaeA	EAE-F and EAE-R	[27]
hlyA	MFSlF and MFSIR	[28]
stx1	SLTI-F and SLTI-R	[10]
stx2	SLTII-F and SLTII-R	[10]
stx2c	VT2v-l and VT2v-2	[29]
stx2d	VT2-cm and VT2-f	[30]
stx2e	Vte-a and Vte-b	[31]
stx2f	128-1 and 128-2	[32]

2.4. Biochemical and PCR identification

For biochemical identification, the isolates were cultured in triple sugar iron agar (TSI), lysine indole motility medium (LIM) and Simmon's citrate agar (SC) (Nishui, Tokyo) at 37 °C for 18 h. The STEC strains were tested for serological analyses by the slide-agglutination test using *E. coli* O and H antigen (Denka-Seiken, Niigata, Japan). Serotyping of untypeable strains was done by K. Tamura in the National Institute of Infectious Disease, Tokyo, Japan. These isolates were tested for the presence of *stx1*, *stx2*, *eae* and *hlyA* genes, using multiplex PCR described [26]. The primers used were shown in Table 1.

3. Results and discussion

The most significant finding of this work at slaughter is that one quarter of cattle infected with STEC O26:H11, O111:H-, O121:H19 or O157:H7 were high-carriers that contained these typical STEC at concentrations of 10^5–10^8 CFU/g (Table 2). The second significant finding is that STEC could be isolated from cattle with a wide range of faecal pH values. Therefore, the presence of a high-carrying animal at the abattoir increases the potential risk of meat contamination during the slaughtering process, regardless of faecal pH.

Recent studies have indicated that O157:H7 colonizes lymphoid follicle-dense mucosa in the terminal rectum and was present predominantly on the surface of the faecal stool [33]. It has been shown that swab culture from this site gives more reliable recoveries of O157:H7 than IMS from the faeces [34]. If these sites were swabbed during collection of the faeces, it is likely that the CFU/g could be higher than actually exists in the faeces. Moreover, non-O157 STEC strains do not share a tropism for the bovine terminal rectum [33] but for the bovine colon [35]. Although these findings suggest that the sampling method may have affected the isolation rates of the different serotypes of STEC, the faecal samples

in this study were collected from the faeces raked out from the rectum of the animals immediately after slaughter.

In this study, all *stx*-positive organisms isolated from cattle were identified as *E. coli* on the basis of biochemical properties. Therefore, a *stx*-PCR-positive faecal sample was defined as STEC positive in this report [6]. The *stx*-PCR-positive faecal samples were detected from 178 (37.2%) of 479 beef cattle, 38 (39.2%) of 97 cull cows and 11 (37.9%) of 29 F1 cattle (Table 2), but these rates were not statistically different ($P > 0.05$) by *t*-test. In recent screening work on slaughtered cattle with *stx*-PCR, rates have varied between 18% and 70% in France [36,37], Australia [26] and Canada [17]. In Japan, rates have also varied between 39% and 79% at breeding or dairy farms [6,38] and were recovered at 41% at a slaughterhouse [38] in Japan. When determined by nested *stx*-PCR, the prevalence was 100% in all dairy groups [6]. The presence of non-pathogenic *E. coli* in faecal samples makes it difficult to isolate STEC strains from the *stx*-PCR-positive faecal samples. To overcome such difficulties, we developed HCl treatment and used a PCR method for detection of the *stx* genes, new chromogenic agar plates highly specific for *E. coli* and CT-SMAC agar plates highly specific for *eae*-positive strains of STEC [9,21]. An acid-shock step was employed to foster selection for and detection of STEC within direct and enriched samples. It is difficult for results from this study to be compared to other studies because of the non-standard methodology. However, it has been confirmed that *E. coli*, including STEC, has resistance to HCl and that HCl treatment is effective for the separation of *E. coli* from other gram-negative bacteria [9,21]. In fact, all 564 strains of *E. coli*, including STEC, survived after being exposed to 0.125 N HCl for 30 s, regardless of differences in serotypes and pathogenicity [9]. The cultures on CT-SMAC and CC agar plates after IMS treatment were also contaminated with high levels of background flora grown in faecal enrichment cultures and a population of bacteria such as *Pseudomonas* spp., *Citrobacter* spp. and *Klebsiella* spp. can be easily masked when streaked on a selective agar. Inclusion of HCl treatment after IMS step in the isolation of O26, O111 and O157 from faecal samples enhanced this sensitivity, as previously described [21]. In this study, 114 STEC strains belonging to 40 different O:H serotypes and non-typeable strains were isolated from 97 animals (15.9%); 74 beef cattle (15.4%), 19 cull cows (19.6%) and 4 F1 cattle (13.8%). It was demonstrated that this method achieved the recovery and isolation of a wide range of STEC serotypes, although no STEC strains were isolated from the remaining 132 *stx*-PCR-positive animals (21.5%). Isolation frequency of STEC among the three cattle groups was not statistically different ($P > 0.05$). The isolation rate of STEC is within the statistical variation of previous farm-based study in Japan [31] which found a value of 19% in cows.

These STEC isolates belonging to 40 different O:H serotypes and five non-typeable strains were separated into three pathogenic groups according to the grouping by Gyles et al. [11] (Table 3). Group 1, which included *stx1*- and/or *stx2*- and *eae*- and *hlyA*-positive strains, consisted of 31 strains isolated from 25 beef cattle (5.2%) and 6 cows (6.2%). This group contained O26, O111, O121 and O157 strains, which occur at moderate to high frequencies in severe human disease. O26:H11: *stx1* was isolated from 20 animals (3.3%), O111:H-: *stx1* and O121:H19: *stx2* from each one animal (0.2%) and O157:H7: *stx1*, *stx2* and *stx1* and 2 from 9 animals (1.5%). Recent reports stated that O26:H11 and O111:H-infection rates in cows of dairy farms were 0 to 1.7% in Australia [39], Germany [40] and the United States [41] but 0.6–2.5% in Japan [6]. Rates in

Table 2
Viable number of STEC strains in bovine faeces

STEC group and serogroup	No of strains	Viable number in faeces (Log 10 CFU/g)														
		Japanese black cattle								Holstein					Hbrid F1	
		<2	2	3	4	5	6	7	8	<2	2	3	4	5	<2	4
Group 1	31	14	1	2	2	3		2	1	4		1		1		
O26:H11	20	10	1	2	1	2		1		3						
O111:HNM	1											1				
0121:H19	1								1							
O157:H7(*stx* 1)	3	1						1		1						
0157:H7 (*stx* 2)	4	3				1										
0157:H7(*stx* 1 and 2)	2				1									1		
Group 2	18	11		1				1		2	1				2	
Group 3	63	37			1		2	3		14		1	1		3	1
Total	112	62	1	3	3	3	2	6	1	20	1	2	1	1	5	1
stx-PCR positive samples	132	106								19					7	

Table 3
Characterization of STEC strains isolated from bovine faeces in a slaughterhouse of Shimane Prefecture, Japan

STEC group and serogroup	No. of strains from animals (positive/examined animals)[a]				No. of strains with the following genes																
					eae						+	+	+	–	–	–	–	NT[a]	NT	NT	
						stx 1						+	+	–	+	+	+	–	+	+	–
							stx 2	*stx* 2c	*stx* 2d	*stx* 2e	+	–	+	+	–	–	+	+	–	+	
	Total	B(74/479)	H(19/97)	HB(4/29)						*hylA*	+	+	+	–	+	–	–	NT	NT	NT	
Group 1	31	25	6	0	31	27	1		1		31	4	23	4							
O26:H11	20	17	3[k]		20	20					20		20								
O1U:H-	1		1		1	1					1		1								
O121:H19	1	1			1				1		1			1							
O157:H7	9	7[bc]	2[l]		9	6	7				9	4	2	3							
Group 2	18	13	2	3	8	17	2				12		7	1		4	4	2			
O55:H-	4	3[c]		1[q]	3	3					3		3					1			
O119:H-	8	5[ef]	1	2[qr]		8					4					4	4				
O161:H-	5	4	1		4	5	2				4		3	1				1			
0165:H-	1	1[g]			1	1					1		1								
Group 3	65	45	17	3	19	40	21	3	6	1	27		15	4	4	8	14	20			
O20:H41	3	2		1		3											3				
O28ac:H4	1	1[h]			1	1					1		1								
O28ac:H-	2	2					2											2			
O44:H15	1	1[b]			1	1					1		1								
O55:H2	1						1											1			
O84:H2	1		1[m]		1	1					1		1								
O84:H-	7	6[dgh]		1[r]	3	5	1				5		2	1		2	2				
091:HUT	1		1[n]			1											1				
O103:H25	1	1			1	1					1		1								
O119:H2	2		2[o]						2									2			
O119:H16	1	1					1											1			
O119:H17	1	1				1											1				
O119:H18	1	6[ef]	3		4	10					8		4			4					
O119:HUT	1	1[i]				1					1					1					
O121:H7	1	1			1		1				1			1							
O124:H19	1	1				1					1					1					
O152:H7	1	1				1											1				
O152:H-	1	1			1		1				1			1							
O160:H21	3	2	1				3											3			
O160:H38	2	2[j]					1	2								1			1		
0160:H-	3	2[j]		1		1	2										1	2			
O161:H2	5	1	4[kmp]		2	2			3		2		2					3			
O161:H19	1		1						1									1			
O161:Haag	1	1					1											1			
O161:HUT	1		I[n]		1	1					1		1								
O165:H8	1	1								1								1			
O168:H8	1	1					1											1			

(*continued on next page*)

Table 3 (*continued*)

STEC group and serogroup	No. of strains from animals (positive/examined animals)[a]				No. of strains with the following genes																							
					eae	*stx 1*		*stx 2*																					
	Total	B(74/479)	H(19/97)	HB(4/29)					*stx* 2c	*stx* 2d	*stx* 2e	*hylA*	+	+	+	−	−	−	−	NT[a]	NT	NT						
O171:H2	3	2	1[o]		1	2			2				1		1		1			1								
O171:H38	1	1				1	1										1											
O171:HNM	1		1[p]			1	1										1											
O211:H7	1	1				1														1								
OR:HUT	3	3[i]			1		3						1			1				2								
ONT:HNT	2	1	1[i]			2											.			2								
Total	114	83	25	6	58	84	30		3	7	1	70	4	45	9	4	12	18	22									
stx-PCS positive samples (non-isolation of STEC strains)	227(132)	178(104)	38(19)	11(7)	NT[s]	166(92)	79(46)		NT	NT	NT	NT											19(9)	147(83)	60(37)			

[a] B: Japanese black cattle, H: Holstein, HB: Hybrid F1 (HXB).
[b–r] Different serotypes were isolated from same cattle.
[s] NT: Not tested.

calves of beef farms were 7.3–19.2% in Japan [13], respectively. This high prevalence of O26:H11 in Japan would reflect the high prevalence of this serotype in the slaughtered cattle in this study. In contrast, O157:H7 has rapidly spread into dairy and beef farms worldwide since this serotype was first described in 1983 [12], although the route of transmission is unclear. Recent reports described O157:H7/-infection rates in slaughtered cattle and/or cows at 1.3–28% in the United States [42,43], 16.1–16.6% in Italy [44], 10.6% in The Netherlands [45], 4.0–4.7% in England [8,13], 0.2% in France [36] and 1.6–8.1% in Japan [5]. According to these reports, although these differences are likely due to differences in culture methods, remarkable differences were found in the rates of recovery of O157:H7 isolates for slaughtered animals in various geographic areas. Human infections involving O157:H7 dramatically increased worldwide since 1983. In Japan, 83% of the human isolates were demonstrated as O157:H7 from 1991 to 1995 and thereafter O26 and O111 have increased [46]. In 2002, the occurrence of O157:H7 decreased to 53% of the human isolates. These findings suggested a close correlation between the human infections and the prevalence of these serotypes in slaughtered animals.

The high-carriage at a concentration of 10^5–10^8 CFU/g of faeces in one third of animals infected with O157:H7 was demonstrated by plating onto CT-SMAC after direct HCl treatment of faeces (Table 2 and Fig. 2), although O157:H7 was recovered at comparatively low frequencies. In 4 of 21 animals infected with O26:H11 and O121:H19, this high rate of carriage in the faeces was also demonstrated. In previous reports [14,41], O157:H7 was found to be more frequently carried by calves and heifers than by adult cattle, and young dairy animals can shed O157:H7 in faeces at 10^3–10^5 CFU/g [14] while beef cattle can shed O26:H11, O111:H- and O157:H7 in faeces at 10^2–10^9 CFU/g [7]. Thus, young animals may be principal reservoirs of group 1. Although these observations were supported by the findings that faecal carriage of O157:H7 in calves was significantly greater than in adult cattle during experimental infection of animals of both ages [19], the present study revealed that adult cattle can also shed a high concentration of group 1 into faeces.

It is well known that STEC prevalence is seasonal and June through August is the time frame in which most human outbreaks and sporadic infections occur in Japan [46]. In this study, almost all STEC-infected animals were also found in the early summer and early fall (June–October) with the peak in June and July. The isolation rates in this warm season were significantly higher than the cold season (November–May) ($P < 0.01$) and those in June and July were dramatically higher than in other months ($P < 0.005$). These findings demonstrate that the carriage of high levels of group 1

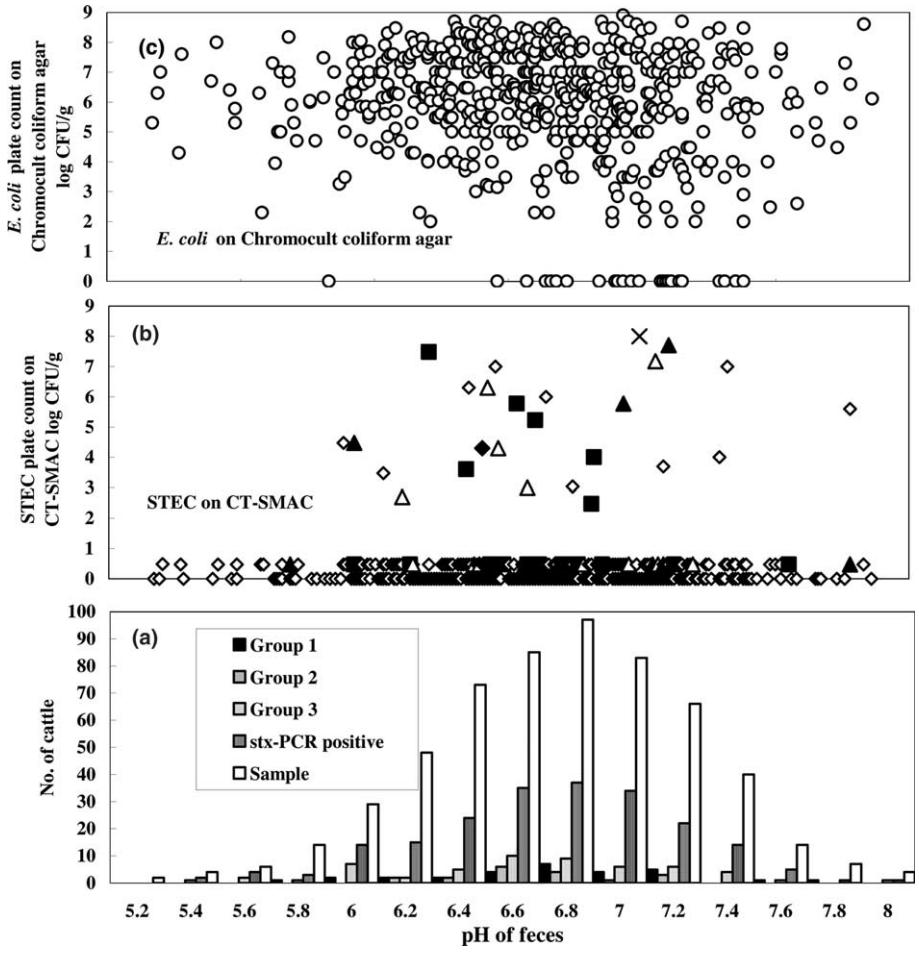

Fig. 2. Relationship between faecal pH and the presence of STEC in the colon of slaughtered adult cattle (a). (b) Plate count of STEC on CT-SMAC, ▲: O157:H7, ■: O26:H11, ◆: O111:H-, ×: O121:H19, △: Group 2, ◇: Group 3; (c): Plate count of *E. coli* on Chromocult coliform agar.

by adult cattle entering the slaughter process in summer is an important risk factor for human infections with STEC group 1.

Groups 2 and 3 and *stx*-PCR positive strains were found in 197 animals (32.5%). Eighteen strains (serotypes O55:H-, O119:H-, O161:H-, and O165:H-) of group 2, which includes mainly *stx1*- and *eae*-positive strains less frequently implicated in human disease, were isolated from 17 animals (2.8%). Sixty-three strains (34 serotypes) of group 3, which included mainly *stx1*-positive strains and have not been implicated in human disease, were isolated from 58 animals (9.6%). The *stx* gene of 10 strains of group 3 were subtyped into *stx2c* (3 strains of O119:H18 and O171:H2), *stx2d* (6 strains of O119:H2, O161:H2 and O161:H19) and *stx2e* (one strain of O165:H8). However, numbers of 10^5–10^8 CFU/g were recovered from only 6 animals (Table 2).

Adult cattle have a fully developed forestomach compartment, the rumen, where the combination of a high-volatile fatty acid content and a low pH inhibits the growth of O157:H7, and the acid-resistant STEC can survive passage through the acidic condition of the stomach [3]. O157:H7 exhibits a novel tropism for terminal rectum in the bovine host [33] and non-O157 for bovine colon [35]. Recently, it was reported that the carrying of O157:H7 in cattle is affected by acid change in their colonic following abrupt dietary changes [16,23]. Although these effects are clear in experimentally inoculated animals exposed to abrupt dietary changes, apparently there are no epidemiological data on cattle herds and slaughtered cattle, showing a correlation among the incidence of STEC group 1 culture-positive cattle, their diet and faecal pH. In this study, the faecal pH of slaughtered cattle from different farms, in which Japanese standard diets for beef cattle and cows contained 30% and 50% grain, respectively, (level varied slightly among farms) was distributed from pH 5.2 to 7.8. The prevalent rates of STEC in these animals also showed a distribution parallel to the faecal pH distribution, regardless of the cattle species (Fig. 2). The concentrations of total *E. coli* in the faeces of most animals were 10^6–10^8 CFU/g of faeces, regardless of faecal pH. Moreover, the prevalence and concentration of STEC group 1 in the faeces were not influenced by faecal pH between

slightly acidic (<pH 6.8) and neutral/slightly basic (pH 7.2⩽, ⩾ pH 6.8) faeces ($P > 0.05$). These findings suggest that members of colonic enterobacterial flora, including total *E. coli* and pathogens such as STEC, are not affected by the faecal pH of cattle fed a normal diet. It also demonstrates that STEC can be isolated from cattle with a wide range of faecal pH values. This phenomenon will be of interest to examine the effect of diet on STEC shedding.

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