NOTE *Public Health*

Comparison of the Prevalence of Shiga Toxin-Producing *Escherichia coli* Strains O157 and O26 between Beef and Dairy Cattle in Japan

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ABSTRACT. With the aim of comparing the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) O157 and O26 between beef and dairy cattle, we collected rectal content samples from 250 beef cattle on 25 beef farms and 250 dairy cows on 25 dairy farms from July through September 2011. STEC O157 was isolated from 16 beef cattle on 7 beef farms, while no STEC O157 was isolated from any dairy farms. This result suggests that the prevalence of STEC O157 is higher in beef cattle than in dairy cattle. STEC O26 was isolated from 1 animal each from beef and dairy cattle herds, and therefore, it was not possible to compare statistically the prevalence of STEC O26 in beef and dairy cattle.

KEY WORDS: beef cattle, dairy cattle, prevalence in summer, STEC O157, STEC O26.

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Shiga toxin-producing *Escherichia coli* (STEC) is an important human foodborne pathogen present worldwide [10]. Shiga toxin (Stx) has been divided into 2 groups, Stx1 and Stx2, each containing antigenically related members. According to the subtyping nomenclature established at the 7th International Symposium on Shiga Toxin (Verocytotoxin) – Producing *Escherichia coli* Infections (Buenos Aires, 10–13 May 2009), Stx variants include 3 Stx1 subtypes (Stx1a, Stx1c and Stx1d) and 7 Stx2 subtypes (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g).

STEC occasionally colonizes the intestinal tracts of cattle and is excreted in the feces. Consumption of STEC-contaminated beef is a cause of foodborne STEC infection [8]. Beef is produced not only from beef cattle but also from dairy catttle. The beef from dairy cattle accounted for approximately 30% of beef production in Japan from 2008 through 2011 [15]. The reduction of fecal STEC shedding in beef and dairy cattle may be the key to prevent human STEC infection. Although many STEC O serogroups cause human STEC infections, STEC O157 has been identified as the cause of most outbreaks of the infection in Japan, whereas STEC O26 has been identified as a causative agent of food poisoning to a lesser extent [8]. The prevalence and characteristics of STEC O157 and STEC O26 in beef and dairy cattle are essential background information for the application of food safety measures along the food chain. We have reported that the prevalence rates of STEC 157 and STEC O26 in 2,436 beef cattle (406 beef farms) were investigated from November 2007 through March 2008, and STEC O157 and STEC O26 isolates were obtained from 218 (8.9%) cattle on 110 (27.1%) farms and from 10 (0.4%) cattle on 7 (1.7%) farms, respectively [12]. In addition, we recently found that although no STEC O26 was isolated from 250 dairy cows, STEC O157 was isolated from 3 dairy cows (1.2%, 3/250) on 1 dairy farm (4.0%, 1/25) from December 2010 through February 2011 [14]. Although the prevalence of STEC O157 might be lower in dairy cows than in beef cattle, a survey should be conducted to confirm this assumption, because the time of collection of samples in these studies might have had an influence on the prevalence of STEC O157 in beef and dairy cattle. In addition, a survey should be conducted in summer, because human STEC infections peak in summer [8].

The objective of the present study was to compare the prevalence of STEC O157 and STEC O26 between beef and dairy cattle in summer.

In total, 50 farms (25 beef and 25 dairy farms) in eastern Japan participated voluntarily in the present study. Regarding the beef farms investigated in the study, the average number of beef cattle raised on each farm was 356 (minimum=30; maximum=2,500). In each of the beef farms, 10 healthy beef cattle, whose scheduled dates of slaughtering were closest among all animals in the farm to the date of sampling, were selected. Regarding the dairy farms investigated in the study, the average number of dairy cows raised on each farm was 141 (minimum=24; maximum=1,000). In each of the dairy farms, 10 healthy lactating dairy cows were selected. From each selected animal, a rectal content sample was collected over the period from July to September 2011. The beef cattle

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Table 1. Characteristics of STEC O157 and O26 strains from beef and dairy farms

Farm		Breed of cattle[a)]	Isolate (serovar)	Virulence gene profile[b)]						Reactivity against VTEC-RPLA		Antimicrobial resistance profile[c)]
				stx$_{1a}$	*stx*$_{2a}$	*stx*$_{2c}$	*eae*	*rfbE*$_{O157}$	EHEC-*hlyA*	Stx1	Stx2	
Beef farm	A	F1	A-1 (O157:H7)	−	−	+	+	+	+	−	+	DSM
		F1	A-2 (O157:H7)	−	−	+	+	+	+	−	+	DSM
	B	JB	B-1 (O157:H7)	−	−	+	+	+	+	−	+	DSM
		JB	B-2 (O157:H7)	−	−	+	+	+	+	−	+	DSM
		JB	B-3 (O157:H7)	−	−	+	+	+	+	−	+	Susceptible
		JB	B-4 (O157:H7)	−	−	+	−	+	+	−	+	DSM
		JB	B-5 (O157:H7)	−	−	+	+	+	+	−	−	DSM
		JB	B-6 (O157:H7)	−	−	+	+	+	+	−	−	Susceptible
	C	JB	C-1 (O157:H7)	+	−	+	+	+	+	+	−	DSM
		JB	C-2 (O157:H7)	+	−	+	+	+	+	+	−	DSM
		JB	C-3 (O157:H7)	+	−	+	+	+	+	+	−	ABPC + DSM
		JB	C-4 (O157:H7)	+	−	+	+	+	+	+	−	ABPC + DSM
	D	F1	D-1 (O157:H7)	+	+	−	+	+	+	+	−	Susceptible
	E	HF	E-1 (O157:H7)	+	−	−	+	+	+	+	−	Susceptible
	F	F1	F-1 (O157:H7)	+	+	−	+	+	+	+	+	Susceptible
	G	F1	G-1 (O157:H7)	−	−	+	+	+	+	−	+	Susceptible
		F1	G-2 (O26:H11)	+	−	−	+	−	+	+	−	Susceptible
Dairy farm	J	HF	J-1 (O26:H11)	+	−	−	+	−	+	+	−	DSM + OTC

a) JB: Japanese Black, HF: Holstein-Friesian, F1: first-generation hybrid of JB and HF.
b) No isolate had stx$_{2d}$, stx$_{2e}$ or stx$_{2f}$.
c) ABPC: ampicillin; DSM, dihydrostreptomycin; OTC, oxytetracycline.

tested included 70 (28.0%) Japanese Black (JB), 20 (8.0%) Holstein-Friesian (HF) and 160 (64.0%) first-generation hybrid (F1) of JB and HF, while all the dairy cows tested were HF. The average age of beef and dairy cattle used in the study in months was 26.2 (minimum=20 and maximum=32) and 53.4 (minimum=20 and maximum=148), respectively. The rectal content samples were kept refrigerated at 4°C for up to 72 hr before analysis.

E. coli O157 and *E. coli* O26 were isolated by the method previously described [12]. *E. coli* O157 and O26 were first characterized in motility and by an agglutination test using anti-H sera (Denka Seiken Co., Ltd., Tokyo, Japan) according to the accompanying instruction manual. The types of the Stx genes (*stx*$_{1a}$, *stx*$_{2a}$, *stx*$_{2c}$, *stx*$_{2d}$, *stx*$_{2e}$ and *stx*$_{2f}$), *eae*, enterohemorrhagic *E. coli* (EHEC) -*hlyA*, *rfbE*$_{O157}$ and *fliC*$_{H7}$ were investigated by PCR analysis using primers reported by Wang *et al.* [16]. *E. coli* O157 strains possessing Stx genes were regarded as STEC O157. The production of Stx1 and Stx2 was confirmed by reverse passive latex agglutination with a Stx detection kit (VTEC-RPLA SEIKEN, Denka Seiken). STEC O157 and STEC O26 isolates obtained in the present study were further characterized by antimicrobial susceptibility testing. The minimum inhibitory concentration (MIC) of 15 antimicrobial agents was determined using the agar dilution method according to the guideline of the Clinical and Laboratory Standards Institute [3]. Fifteen antimicrobial agents tested were ampicillin (ABPC), cefazolin (CEZ), ceftiofur (CFT), dihydrostreptomycin (DSM), gentamicin (GM), kanamycin (KM), apramycin (APM), oxytetracycline (OTC), bicozamycin (BCM), chloramphenicol (CP), colistin (CL), nalidixic acid (NA), enrofloxacin (ERFX), trimethoprim (TMP) and fosfomycin (FOM). ABPC, GM, KM, OTC, CP, NA and TMP were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); CEZ, DSM, CL and FOM from MP Biomedicals Japan Inc. (Tokyo, Japan); CFT from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan); BCM from Food and Agricultural Materials Inspection Center (Saitama, Japan); and APM and ERFX from LKT Laboratories, Inc. (St. Paul, MN, U.S.A.). *Enterococcus faecalis* ATCC29212, *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC29213 were used as the quality-control strains. Concentrations of all agents tested ranged from 0.125 to 256 mg/*l*. The resistance breakpoints adopted by previous studies [4, 11, 13] were used.

STEC O157 was isolated from 16 (6.4%) beef cattle on 7 (28%) beef farms, but not obtained from any dairy cows tested (Table 1). All the strains were motile and identified as STEC O157:H7 using anti-H sera and PCR analysis of *fliC*$_{H7}$. The prevalence of STEC O157 was statistically (P=0.0001, chi-square test with Yate's correction for continuity) higher in beef cattle than in dairy cattle. Thirteen STEC O157 strains, except for 3 strains (B-4, B-5 and B-6), had *eae*, EHEC-*hlyA* and *rfbE*$_{O157}$ and produced Stx1 and/or Stx2. Stxs were not detected in 2 STEC O157 strains, B-5 and B-6, that possessed only *stx*$_{2c}$. It has been reported that VTEC-RPLA could react with both Stx2a and Stx2c, but that it was 30-fold less sensitive to purified Stx2c than to purified Stx2a [9]. Therefore, even if these 2 STEC O157 isolates produce Stx2c, it may not be detected. Two STEC O26 isolates were obtained from one each of beef and dairy cattle. While the number of STEC O26 isolate from beef cattle was the same as that from dairy cattle, the number was too small for robust statistical analysis. The 2 STEC O26 isolates were motile and identified as STEC O26:H11 using

anti-H sera. Both the two STEC O26 isolates had *eae* and EHEC-*hlyA* and produced Stx1. Considering that the beef from beef cattle and that from dairy cattle accounted for approximately 70% and 30%, respectively, of beef production in Japan from 2008 through 2011 [15], the reduction of the prevalence of STEC O157 in beef cattle is regarded more effective than that in dairy cattle in decreasing human STEC O157 infections.

In our previous study [12], the presence of dogs and/or cats on a beef farm was significantly associated with the prevalence of STEC O157 in beef farms, while there was no statistical association between the prevalence of STEC O157 and the breed of the cattle. In the present study, the risk factors for the prevalence of STEC O157 were evaluated with the same questionnaire to the beef farmers as the previous study [12]. The presence of dogs and/or cats on a beef farm was not statistically associated with the prevalence of STEC O157 in beef farms. Surveys on the prevalence of STEC O157 in dogs and cats are desirable to clarify contribution of the presence of dogs and cats in beef farms to the prevalence of STEC O157 in cattle. With regard to the breed of the cattle, STEC O157 strains were isolated from 10 (14%) of 70 JB cattle, 1 (5%) of 20 HF cattle and 5 (3%) of 160 F1 cattle, and there was a statistically significant association (P=0.004, chi-square test with Yate's correction for continuity) between the prevalence of STEC O157 and the breed of the cattle (between JB cattle and F1 cattle). However, 10 JB cattle positive for STEC O157 were from only 2 beef farms (B and C), and some STEC O157 isolates from the respective farms were genetically and phenotypically identical. Two STEC O157 strains from two F1 cattle in farm A were also genetically and phenotypically identical to each other. The results suggest that once an STEC O157 strain is excreted from 1 cattle, it spreads to other cattle on the farm, as reported by the previous study [12]. The within-farm spread of STEC O157 may influence the association between the prevalence of STEC O157 and the breed of the cattle. Further studies are needed to identify risk factors associated with the prevalence of STEC O157 in beef farms.

It has been known that STEC O157 is excreted at higher frequency in the warmer months and at lower frequency in the cold months [2, 6, 7]. Although we anticipated that the prevalence of STEC O157 would be higher in beef cattle than that (8.9%) in our previous study [12], the rate in the present study and that in the previous study were very similar. Alam *et al*. [1] has reported that the prevalence of *E. coli* O157 in beef cattle was not season-dependent. Ezawa *et al*. [5] has reported that the seasonal prevalence of STEC O157 in dairy cattle varied according to the year investigated. Since our previous study, 4 years have passed. If there is a decreasing trend in the prevalence of STEC O157 in beef cattle, the 2 studies conducted 4 years apart and in 2 different seasons are not sufficient for determining whether seasonal variation exists. A study throughout the year is therefore needed to get clear answer to this question.

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