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SURVEY OF SHIGA TOXIN-PRODUCING *Escherichia coli* (STEC) IN BEEF AND DAIRY CATTLE AT FARMS AND SLAUGHTERHOUSE

Kunihiro Shinagawa[1], Masahiko Kanehira[1], Katsuhiko Omoe[1], Ibuki Matsuda[1], and Shunji Sugii[2]

[1]Microbiology Laboratory, Department of Veterinary, Faculity of Agriculture, Iwate University, Morioka, 020-0088, Japan

[2]Microbiology Laboratory, Department of Veterinary, Faculity of Agriculture, Osaka Prefecture University, 599-8531, japan

Background:

Serious outbreaks of food poisoning associated with *Escherichia coli* O157:H7 has occurred in Japan since June in 1996. Although food poisoning is a serious social problem, the source and route of infection have not yet been fully understood. Since Shiga toxin-producing *E. coli* (STEC) has often been isolated from rectal feces of beef and dairy cattle, cattle is supposed to be one of the STEC sources at the present time. The main serotypes of STEC in human infection are known to be O157, O26, and O111, etc.

Objectives:

In the present study, we made attempts to detect genes for Shiga toxin (STx)1 and STx2 of *E. coli* in rectal feces from cattle at farms and a slaughterhouse by polymerase-chain reaction(PCR) and to isolate *E. coli*(serotypes O157, O26, and O111) by immunomagnetic separation (IMS) to see whether or not cattle is a potential STEC source.

Materials and Methods:

Sampling : 854 fecal specimens were obtained from 4 farms(A-D) and 1 slaughterhouse. The specimens obtained from farms were collected during 3 seasons (winter, spring, and autumn), whereas those obtained from the slaughterhouse were collected from November of 1998 to July of 1999.

Culture methods : Fecal specimens (1 g) were cultured in mEC broth with Novobiocin for selective enrichment, and inoculated into Tryptosoy enrichment medium.

PCR : For detection of STx genes in culture of fecal specimens by PCR, we used PCR primer pairs for STx1 and STx2 (Takara Shuzou Co. Ltd.).

Isolation of STEC(O157, O26, and O111) : STx-positive specimens were inoculated into mEC broth with Novobiocin. Preenriched sample was mixed with immunomagnetic beads sensitized with antibodies against *E. coli* O157, O26, and O111. The complex of bead-bacterium was washed and plated onto CT-SMC agar and CHROM agar O157. Colonies (5-6 colonies) grown on CT-SMC agar and CHROM agar O157 were used for testing agglutination with antisera to *E. coli* O157, O26, *and* O111. After identification of serotype of *E. coli*, STx genes were also detected by PCR. When STx gene(s) was detected, STEC was supposed to be infected with the cattle.

Results and Discussion:

Detection of STx genes in feces by PCR : Of 169 fecal specimens collected at farm A tested, STx genes were detected 68.0% (115/169) of the samples. STx1 gene, STx2 gene, and both STx1 and STx2 genes were detected in 39, 22, and 54 samples, respectively. STx genes were also found in 41.9%, 22.2%, and 12.2% of the fecal samples collected at farms C, D, and B, respectively. On the other hand, STx genes were detected in 39.2% (144/367) of fecal specimens isolated from the slaughterhouse. STx1 gene, STx2 gene, and both STx1 and STx2 genes were identified in 39, 50, and 55 samples, respectively. Of a total of 854 fecal samples tested, STx1 gene, STx2 gene, and both STx1 and STx2 genes were 11.4% (97), 11.8% (101), and 16.2% (138) of the fecal specimens, respectively (Table 1). At the present time, it is not known whether the fecal specimens contained either a single STEC or two different STEC when genes for both STx1 and STx2 were detected.

Detection of STEC in feces of cattle at different ages and kinds : STEC was detected in 40.0%, 60.3%, and 77.0% of fecal samples from calves in winter (February), spring (May), and autumn (October), respectively ,whereas that was detected in 60.3%, 21.5%, and 8.8% of samples from heifers and adult cattle, respectively. Taken together, STEC was detected in 60.0% of fecal samples from calves, whereas that was detected in 19.4% from heifers and adult cattle. There is statistically significant difference (p=0.05)between heifers and adult cattle.

STEC was detected in 49.9%, 74.4%, 56.6%, 57.1%, 61.1%, and 62.5% of the fecal specimens from calves aged at 0 to 2 months, 2 to 4 months, 4 to 6 months, 6 to 8 months, 8 to 10 months, and 10 to 12 months, respectively.

Of fecal specimens collected at 4 farms, STEC was detected in 79.6%, 50.0% , and 32.8% of fecal specimens from Japanese black beef cattle "Kuroge", F1 of Japanese black beef cattle and Holstein, and Holstein,

respectively. On the other hand, STEC was also detected in 56.2%, 40.7% , 35.8% and 18.9% of fecal specimens from Japanese beef black cattle, F1 of Japanese black beef cattle and Holstein, Japanese short horn cattle "Tan-kaku", and Holstein, respectively (Table 2).

From these findings, there is significant difference in detection of STEC in fecal specimens from Japanese black beef cattle and other cattle.

Detection of STEC in feces of cattle at 4 farms : To study how long STEC is infected with cattle, fecal specimens from the same cattle at different time were tested for STEC. Of fecal specimens collected at 4 farms (A-D). At farm A, STEC with the STx gene(s) was always detected in 3 out of 7 fecal specimens collected in February, April, and May. STEC was also detected in 1 out of 7 fecal specimens collected in February and May, whereas that was in 1 out of 7 samples in April and May. In addition to these, all STEC with different STx genes were detected in every month. Similar findings were also obtained with fecal specimens from farms B, C, and D. STEC with the same STx gene(s) was not detected in these three months. From these findings, STEC infection in cattle may not continue for more than 5 months.

By use of PCR, 140 fecal specimens collected at 4 farms were STEC-positive. Of 140 STEC tested, 3.6% (5/140) was identified as STEC O157, whereas 0.7% (1/140) was identified as STEC O26. On the other hand, 2.1% (3/144) was identified as STEC O157 in 144 STEC isolated from fecal specimens at a slaughterhouse. STEC O111 was not detected in fecal specimens at farms and slughterhouse. Further studies will be needed although STEC O157, O26, and O111 associated with human infection were not highly detected in fecal specimens of cattle in the present study.

Conclusions:

1. STEC was found to be highly infected with cattle at farms and slaughterhouse.
2. Sixty percent of calves aged less than 1 year were highly infected with STEC, whereas 19.1% of calves aged older than 1 year was infected.
3. STEC was most highly infected with Japanese black beef cattle.
4. STEC infection in cattle may be short (less than 5 months).
5. STEC O157, O26, and O111 associated with human food poisoning were not often detected in fecal specimens of cattle.

Table 1. Detection of STx genes in feces of cattle, foul water, and compost by PCR

Samples		No. of samples	No. of STx positive samples detected by PCR			
			STx 1	STx 2	STx 1&2	Total (%)
Feces of cattle						
Farm	A	169	39	22	54	115(68.0)
	B	82	-	7	3	10(12.2)
	C	74	4	12	15	31(41.9)
	D	162	15	10	11	36(22.2)
Slaughterhouse		367	39	50	55	144(39.2)
Total		854	97	101	138	336(39.3)
Foul water (Farms & Slaughterhouse)		17	2	1	4	7(41.2)
Compost (Farms)		8	2	2	-	4(50.0)

Table 2. Detection of STx genes in feces of at defferent kinds

	Kinds of cattle		No. of specimens	No. of STx positive samples detected by PCR			
				STx1	STx2	STx1&2	Total(%)
Farms	Dairy cattles	Holstein	393	39	34	56	129(32.8)
	Beef cattles	"Kuroge"	54	10	10	23	43(79.6)
		F1	40	9	7	4	20(50.0)
Slughterhouse	Dairy cattles	Holstein	111	6	9	6	21(18.9)
	Beef cattles	"Kuroge"	146	24	23	35	82(56.2)
		F1	54	3	15	4	22(40.7)
		"Tan-kaku"	53	6	3	10	19(35.8)