Isolation and Characterization of Shiga Toxin-Producing *Escherichia coli* O157:H7 from Beef, Pork and Cattle Fecal Samples in Changchun, China

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ABSTRACT. Meat samples and fecal specimens from adult cattle were collected in Changchun, China and were examined for presence of Shiga toxin-producing *Escherichia coli* (STEC) serogroup O157. STEC O157 strains were isolated from 2 (5%) of 40 beef, 1 (3.3%) of 30 pork, and 3 (1.7%) of 176 adult cattle fecal samples. The strains belonged to phage types (PT) 4, 8, or 47. Two beef strains and a strain previously isolated from a patient in Shandong, China, were PT-4 and showed a similar PFGE pattern, suggesting the possibility of food-borne transmission. It is suggested that cattle are a reservoir of STEC O157:H7 and meat products are contaminated by this pathogen in Changchun, China as well as in other countries.

KEY WORDS: China, *Escherichia coli*, O157.

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) serogroup O157 (O157:H7 or O157:NM [non-motile]) has been associated with a variety of human diseases, including mild diarrhea, hemorrhagic colitis, and diarrhea-associated form of the HUS since it was first described in 1982 [6]. Humans become infected with STEC O157 following the consumption of contaminated foods or water or by direct transmission from infected humans or animals [1, 3, 6]. Undercooked ground beef and raw milk have most often been implicated in food-borne infections. Since healthy domestic animals, in particular, ruminants like cattle, sheep, and goats can harbor STEC O157 in their feces, they have been regarded as natural reservoirs of this pathogens [2]. In China, outbreaks due to STEC O157 have not been reported. Although several sporadic cases of STEC O157 infection have been reported [10, 12, 17, 18], the sources of in these cases have not been defined. To assess the significance of meat and cattle as the vehicle or the reservoir, beef, pork and cattle fecal samples were collected in the Changchun area and examined for STEC O157. The isolates were characterized to assess their diarrheagenicity and were analyzed for epidemiological comparison.

In the period from September to November 1996, beef and pork samples were collected at several free markets of Changchun. The samples were brought to the laboratory and examined for STEC O157 within 1 hr. Fecal samples from adult cattle were collected at two farms and two slaughterhouses of Changchun between May and August 1997. The swabs very heavily coated with feces were transported immediately to laboratory at ambient temperature. Upon arrival at the laboratory, the swabs were analyzed either immediately or held at 4°C no longer than 48 hr before analysis.

A 25 g of portion of each beef and pork sample was aseptically removed and added to 225 ml of modified trypticase

soy broth (mTSB) in a 500-ml flask. The mTSB enrichment medium was composed of (per liter) Trypticase soy broth (TSB; 30 g; Sigma Chemical Co., St. Louis, Mo., U.S.A.), bile salt 3 (1.5 g; Difco Laboratories, Detroit, Mich., U.S.A.), dipotassium phosphate (1.5 g), and novobiocin (20 mg; Sigma Chemical Co., St. Louis, Mo., U.S.A.). The mTSB enrichment medium was prepared by the method previously described [5]. For cattle fecal samples, each sample swab was added to 225 ml of mTSB in a 500-ml flask. The samples were incubated for 18 to 24 hr at 37°C. Each enrichment culture was spread plated onto sorbitol MacConkey agar (SMAC) [13]. SMAC medium was prepared as follows with MacConkey agar base (Difco Laboratories, Detroit, Mich., U.S.A.), which is identical to MacConkey agar but contains no lactose. MacConkey agar base (40.0 g) and D-sorbitol (10.0 g) (Difco) were mixed in 1 liter of distilled water, autoclaved, and poured into plates for use. The SMAC plates were incubated at 37°C for 24 hr. Up to 10 non-sorbitol fermenting (NSF) colonies were selected from each SMAC plate and streaked onto eosin methylene blue agar (EMB) (Difco Laboratories, Detroit, Mich., U.S.A.). The EMB plates were incubated at 37°C overnight. Those isolates with a typical *E. coli* metallic sheen on EMB were identified by biochemical tests. The isolates were confirmed to be of serotype O157:H7 or serotype O157:NM with an *E. coli* O157 latex test kit (Oxoid Ltd., Basingstoke, Hampshire, UK) and *E. coli* O- and H-specific antisera (Denka Seiken Co., Ltd., Tokyo, Japan).

The presence of *stx1*, *stx2*, *eaeA*, *eae* O157, or *flc h7* was examined by PCR as described previously [7, 8, 15]. Table 1 describes oligonucleotide primers used in the PCR reaction mixture. The primers used to detect the presence of *stx2*, *stx2vha*, *stx2vha* gene sequences are also listed in Table 1. Stx2 genotype of *E. coli* isolates were determined according to the method of Tyler *et al.* [16]. In brief, 10 µl

Table 1. Oligonucleotide primers used in the study

Primers	Oligonucleotide sequence (5'-3')	Product size (bp)	Target genes	Reference
VT1a VT1b	GAAGAGTCCGTGGGATTACG AGCGATGCAGCTATTAATAA	130	<i>stx1</i>	15
VT2a VT2b	TTAACCACACCCACGGCAGT GCTCTGGATGCATCTCTGGT	346	<i>stx2</i>	15
AE9 AE10	ACGTTGCAGCATGGGTAAGTC GTCCACTTTGACAACGGCTAG	815	<i>eaeA</i>	7
AE19 AE20	CAGGTCGTCGTGTCTGCTAAA TCAGCGTGGTTGGATCAACCT	1087	<i>eaeO157</i>	8
FLICH7-F FLICH7-R	GCGCTGTCGAGTTCTATCGAGC CAACGGTGACTTTATCGCCATTCC	625	<i>fliCh7</i>	8
VT2-c VT2-d	AAGAAGATGTTTATGGCGGT CACGAATCAGGTTATGCCTC	285	<i>stx2</i> , <i>stx2vha</i> , <i>stx2vhh</i>	16
VT2v-1 VT2v-2	CATTACACGATAAAAGTGGCC GGGTGCCTCCCGGTGAGTTC	385	<i>stx2vha</i> , <i>stx2vhh</i>	16

aliquots of amplification reaction mixture recovered after the PCR using the VT2-c and VT2-d primers were subjected to restriction endonuclease digestion with *Hae*III, *Rsa*I, or *Nci*I (New England BioLab Inc., Beverly, MA, U.S.A.) as recommended by the manufacturer. The restriction endonuclease digestion of this amplicon permitted prediction of specific *stx2*, *stx2vha* and *stx2vhh* on the basis of predetermined restriction fragment length polymorphisms.

Isolates were grown overnight at 37°C with shaking (120 rpm) in 5 ml Casamino acid-yeast extract medium (Difco Laboratories, Detroit, Mich., U.S.A.). A 0.5 ml portion of each culture was transferred into an Eppendorf tube and 25 µl of 5,000 U polymyxin B was added. After incubation at 37°C for 30 min, the supernatants were obtained by centrifuging the cultures at 15,000 rpm for 2 min. Stx production was determined by the reversed-passive latex agglutination with VTEC-RPLA kit (Denka Seiken) according to the manufacturer's instructions. Further, STEC O157 isolates were characterized by the pattern of adherence to HEp-2 cells as described previously [14].

Isolates confirmed as STEC O157 were phage typed at the Laboratory for Enteric Pathogens, Central Public Health Laboratory, London, UK by the scheme of Khakhria *et al.* [11]. As a molecular epidemiological method pulsed field gel electrophoresis (PFGE) was performed according to the method of Izumiya *et al.* [9]. *Xba*I (Takara Shuzo, Otsu, Shiga, Japan) was used as the restriction endonuclease. Digested DNAs were separated by electrophoresis through a 1% agarose gel by using a CHEF DR II apparatus (Bio-Rad, Hercules, CA, U.S.A.) in 0.5 × Tris-borate-EDTA buffer at 14°C with a voltage of 200V. A linearly ramped switching time from 4 to 8 s was applied for 11 hr and then a linearly ramped switching time from 8 to 50 s was applied for 9 hr.

In 1996, STEC O157 strains were isolated from 2 (5%) of 40 beef samples and 1 (3.3%) of 30 pork samples (Table 2). Food products have been the most frequently implicated source of STEC O157 in outbreaks and sporadic cases. Beef and other red meats have been considered to be highest risk foods. In an early study, STEC O157 was isolated from 3.7% of retail beef, 1.5% of pork samples in the United States and Canada [5]. This present study demonstrated that meat products were contaminated by this pathogen in a high percentage in the Changchun area. If the immunomagnetic separation method had been used, this rate would have been higher because this method increases the recovery of STEC O157 from the selective enrichment broth [4].

In 1997, STEC O157 strains were isolated from 3 (1.7%) of 176 fecal samples from adult cattle (Table 2). In addition a strain of STEC O8 was isolated from one of these samples. Only the NSF isolates were examined in this study. These STEC strains were only isolated from 146 fecal samples of beef cattle at slaughterhouses, and no STEC strain was recovered from 30 fecal samples from cattle on farms. The origins of the animals and the origin of meat positive for STEC O157 could not be traced. Reported estimates of the prevalence of STEC O157 in North America and European cattle range from 0 to almost 10% [1]. Our results suggest that cattle are a reservoir of STEC O157 in China as well as in other countries.

Properties of the STEC O157 strains are presented in Table 2. All STEC O157 isolates from cattle, beef and pork possessed *stx* and *eae* genes. The organisms produced Stx and adhered to HEp-2 cells in a localized manner which is characteristic of wild strains possessing LEE [14]. The strains were definitely potential human pathogens.

Three phage types (PT-4, 8 and 47) were identified

Table 2. Characterization of STEC O157 isolates from beef, pork and fecal specimens

Isolates	Virulent genes				RPLA titer		Phage type	Adherence to HEp-2 cell	Origin
	<i>stx1</i>	<i>stx2</i>	<i>stx2vha</i>	<i>eae</i>	Stx1	Stx2			
96001	+	+	-	+	80	160	4	LA ^{b)}	Beef
96005	+	+	-	+	80	160	4	LA	Beef
96009	-	+	+	+	-	2560	47	LA	Pork
97063	+	+	-	+	160	1	RDNC ^{a)}	LA	Cattle feces
97094	+	+	+	+	160	1	8	LA	Cattle feces
97168	+	+	+	+	80	1	8	LA	Cattle feces
94H	+	+	-	+	640	320	4	LA	Human feces

a) RDNC: this culture Reacts with typing phages but Does Not Conform to a recognized pattern.

b) LA: localized adherence.

among the 7 food, animal and human STEC O157 isolates; one of the STEC O157 from cattle feces did not conform to a recognized pattern (Table 2). A strain of STEC O157 that was isolated from a patient in 1994 was compared with the other strains by PFGE. No identical PFGE pattern was recognized in the isolates tested, but a very similar pattern was seen with two beef isolates 96001, 96005 and a human isolate 94H (Fig. 1). They all belonged to PT-4, and PT-4 is one of the most common phage types of human isolates in North America[11]. The similar PFGE patterns and identical phage type between two beef isolates and one human isolate suggest that they might have a relationship. It is likely that meat products have played a role in the transmission of STEC O157 to human in China.

PT-4 and 8 have been frequently isolated from both human and non-human sources in many other countries, but PT-47 was originally reported to be the type unique to Japan. Khakhria *et al.* [11] reported that some phage types were common worldwide, some were found only in definite geographical area. Types 49, 50, 51, and 52 were isolated in the U. K. but not in Canada. Within Canada, types 36, 38 and 53 were observed only in British Columbia and types 55 and 58 only in Alberta. To our knowledge, however, PT-47 has not been detected, even in Japan, after the report of Khakhria *et al.* [11]. We found it among only 7 isolates, so it might be a type restricted in East Asia area.

To our knowledge this is the first report of isolation of STEC O157 from meat and cattle feces in China. It can be concluded that cattle are natural hosts of STEC O157 in China with beef and pork contaminated by this pathogen in the Changchun area. Further investigations should be performed throughout China to define the association between this pathogen in cattle and meat and as a cause of human disease.

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REFERENCES

1. Armstrong, G.L., Hollingsworth, J. and Morris, J.G.Jr. 1996.

M 1 2 3 4 5 6 7 M

Fig. 1. PFGE of STEC O157:H7 strains isolated from Changchun, China. Lanes M, molecular weight markers (lambda ladder standard); 1, STEC O157:H7 96001; 2, STEC O157:H7 strain 96005; 3, STEC O157:NM strain 96009; 4, STEC O157:H7 97063; 5, STEC O157:H7 strain 97094; 6, STEC O157:H7 strain 97168; 7, STEC O157:H7 strain 94H.

- Epidemiol. Rev.* **18**: 29–51.
2. Beutin, L., Geier, D., Steinruck, H., Zimmermann, S. and Scheutz, F. 1993. *J. Clin. Microbiol.* **31**: 2483–2488.
3. Boyce, T.G., Swerdlow, D.L. and Griffin, P.M. 1995. *New. Engl. J. Med.* **333**: 364–368.
4. Chapman, P.A., Siddons, C.A., Zadik, P.M. and Jewes, L. 1991. *J. Med. Microbiol.* **35**: 107–110.
5. Doyle, M.P. and Schoeni, J.L. 1987. *Appl. Environ. Microbiol.* **53**: 2394–2396.
6. Griffin, P.M. and Tauxe, R.V. 1991. *Epidemiol. Rev.* **13**: 60–98.
7. Gannon, V.P.J., Rashed, M., King, R.K. and Thomas, E.J.G. 1993. *J. Clin. Microbiol.* **31**: 1268–1274.

8. Gannon, V.P.J., D'Souza, S., Graham, T., King, R.K., Rahn, K. and Read, S. 1997. *J. Clin. Microbiol.* **35**: 656–662.
9. Izumiya, H., Terajima, J., Wada, A., Inagaki, Y., Itoh, K.I., Tamura, K. and Watanabe, H. 1997. *J. Clin. Microbiol.* **35**: 1675–1680.
10. Kain, K.C., Barteluk, R.L., Kelly, M.T., Xin, H., Hua, G.D., Yuan, G., Proctor, E.M., Byrne, S. and Stiver, H.G. 1991. *J. Clin. Microbiol.* **29**: 90–95.
11. Khakhria, R., Duck, D. and Lior, H. 1990. *Epidemiol. Infect.* **105**: 511–520.
12. Li, J., Zhou, G.Q., Wen, X.O., Cui, S.Y., Meng, W., Wang, Y.L. and Sun, Q.H. 1994. *Chinese J. Zoonoses* **10**: 23–25. (In Chinese.)
13. March, S.B. and Ratnam, S. 1986. *J. Clin. Microbiol.* **23**: 869–872.
14. Nishikawa, Y., Scotland, S.M., Smith, H.R., Willshaw, G.A. and Rowe, B. 1995. *Microbial. Pathogenesis* **18**: 223–229.
15. Pollard, D.R., Johnson, W.M., Lior, H., Tyler, S.D. and Rozee, K.R. 1990. *J. Clin. Microbiol.* **28**: 540–545.
16. Tyler, S.D., Johnson, W.M., Lior, H., Wang, G. and Rozee, K.R. 1991. *J. Clin. Microbiol.* **29**: 1339–1343.
17. Xu, J.G., Quan, T.S., Xiao, D.L., Fan, T.R., Li, L.M., Wang, C.A., Li, W. and Liu, H.M. 1990. *Curr. Microbiol.* **20**: 299–304.
18. Zhang, Z.G., Li, L., Zhang, B.A., Li, Y.F., Hong, Q.Y. and Feng, S.X. 1994. *Chinese J. Med. Lab. Sci.* **17**: 235 (in Chinese).