



ORIGINAL ARTICLE

Prevalence of Farm and Slaughterhouse Workers Carrying Shiga Toxin-Producing *Escherichia coli* in Korea

Sahyun Hong^a, Seung Eun Song^a, Kyung Hwan Oh^a, Seung Hak Cho^a,
Seong Han Kim^a, Seok ju Yoo^b, Hyun Sul Lim^b, Mi Sun Park^{a,*}

^aDivision of Enteric Bacterial Infections, Korea National Institute of Health, Osong, Korea.

^bDongguk University, College of Medicine, Gyeongju, Korea.

Received: July 4, 2011

Revised: September 26, 2011

Accepted: October 27, 2011

KEYWORDS:

farmer,
Shiga toxin,
slaughter,
STEC,
stx

Abstract

Objectives: The aim of this study was to investigate the distribution of Shiga toxin (*Stx*) gene-positive stool samples from dairy farmer and slaughterhouse workers in Gyeonggi-Do province.

Methods: A total of 621 samples from healthy farmers and 198 samples from slaughterhouse workers were screened by polymerase chain reaction (PCR) for Shiga toxin-producing *Escherichia coli* (STEC) infection on stool samples.

Results: The PCR product of *Stx*-encoding genes was detected in 21 (3.4%) of 621 farmers and 15 (7.6%) of 198 slaughterhouse workers' stool samples. Distribution of the *Stx* PCR positive workers by age increment revealed an increase in STEC infection with age increment in both workers. Distribution of the *Stx* PCR positive workers by working years revealed an increase in STEC infection with working years in farmers.

Conclusion: These results of the study show that slaughterhouse workers are at higher risk of STEC infection than farmers. In addition, slaughterhouse workers have a more potential source of food contamination of STEC and transmission.

1. Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) comprises a subset of serotypes of Shiga toxin (*Stx*)-producing *E. coli* (STEC) that has been associated with bloody diarrhoea and hemolytic uremic syndrome (HUS) in

industrialized countries [1,2]. The majority of the EHEC infections worldwide are caused by strains of serotype O157:H7 [3,4].

Stxs and Vero toxins (VTs) are considered to be the major virulence factors of EHEC and comprise a family of structurally related cytotoxins with similar biological

*Corresponding author.

E-mail: pmsun63@korea.kr

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

activity. The two main groups consist of *Stx1* and *Stx2*. *Stx1* is nearly identical to the toxin of *Shigella dysenteriae* type 1 and *Stx2*, which shares less than 60% amino acid sequence with *Stx1* [5]. Whereas *Stx1* exhibits only slight sequence variations, several variants of *Stx2* with altered antigenic or biologic characteristics have been described [6,7].

Most of the reports have been concerned mainly with the associations between the virulence factors of STEC, disease, and transmission [4,8]. In Korea and elsewhere, cattle are considered to be a main reservoir of STEC. In national surveillance programs, the incidence of STEC-related diseases and the isolation rates of STEC have been reported, but there have been no reports concerned of STEC surveillance in dairy farmers and slaughterhouse workers in same region. Gyeonggi-Do province is nearest dairy farm region to Seoul, the capital city of Korea. Dairy farm and Slaughterhouse in Gyeonggi-Do province shared 41.0% dairy farm (3143/7657) and 12.8% Slaughterhouse (10/78) in Korea [9].

Dairy farmers are in direct contact with dairy cattle nearly every day and have an increased possibility of becoming infected with STEC. Also, slaughterhouse workers contact cattle every day and have high a possibility of STEC infection. Therefore, the purpose of this study was to assess the prevalence of STEC both dairy farmers and slaughterhouse workers to evaluate the relationship between STEC infection and work fields as well as to determine STEC infection as a potential source of food contamination and transmission.

2. Materials and Methods

2.1. Stool sample collection

Fecal samples of dairy farmers in November 2008 through April 2009 and 621 samples were collected. For the slaughterhouse workers, a total of 198 fecal samples from October 2007 to April 2008 were collected. We divided the slaughterhouse workers into four categories: inspector, slaughterer, residual products handler, and livestock hygiene controller.

2.2. DNA manipulations and genetic techniques

A loopful of human stool sample was directly inoculated into 3 ml of Tryptic Soy Broth (Oxoid, Basingstoke, Hampshire, United Kingdom) for enrichment and incubated overnight at 37°C with shaking. After incubation, the enriched broth culture was used to isolate chromosomal DNA. Chromosomal DNA was purified using the GenomicPrep Cell and Tissue DNA isolation kit (Amersham Biosciences, Netherlands). All polymerase chain reactions (PCRs) were performed with the Expanded High Fidelity Polymerase System (Roche) or *Taq* polymerase (Takara, Japan) according to the manufacturer's instructions. The primers used for amplification are listed in Supplemental Table 1.

2.3. Statistical analysis

The data collected were evaluated using the SPSS 14.0 statistical package. The distribution of *stx* PCR positive samples from workers by the field of work, age and working year was analyzed using the Chi-square test.

3. Results

3.1. Screening for *Stx*-encoding genes

Of the 621 farmers who underwent stool examination results related to EHEC infection by PCR examination, 21 patients (3.4%) were positive. Of the 198 slaughterhouse workers, 15 workers (7.6%) were positive (Table 1). In an EHEC infection survey by sex-related test in farmers, the *stx* PCR test was positive in 15 out of 396 male farmers (3.8%), and 6 people out of the 225 female farmers (2.7%).

In slaughterhouse workers, 198 stool samples were tested; 14 male workers (7.7%) were positive and only 1 woman out of 31 (0.5%) was positive.

3.2. Distribution of age of *Stx* positive workers

We divided the age of workers into five categories: below 30, 30 to 39, 40 to 49, 50 to 59, and over 60 years, and surveyed the age distribution of *Stx* positive workers. Results of farmers revealed that within the groups 40 years or older—'40 to 49' and '50 to 59'—they shared the rate of 1.0% and 4.9% positive and the 'over 60' group showed the highest rate of 5.8%, and the difference among age classes was significant based on the Chi-square test ($p = 0.01$; Table 2). In slaughterhouse workers in the '40 to 49' and '50 to 59' groups, they shared the rate of 2.5% and 3.0% and the 'over 60' group showed a rate of 2.0% with a Chi-square test of 0.118. Thus, the results showed that as the worker age increased, the percentage of *Stx* positivity increased in the farmers.

3.3. Distribution of working year of *Stx* positive workers

We also surveyed the distribution pattern of the working year of *Stx* positive workers on the basis of

Table 1. Distribution of stool samples showing *Stx* positive by polymerase chain reaction compared with work fields and sex

Sex	Farmer		Slaughterer	
	Positive (%) [*]	Negative (%)	Positive (%) ^{**}	Negative (%)
Male	3.8	61.3	7.7	83.6
Female	2.7	35.2	0.5	16.4
Total	3.4	96.6	7.6	92.4

^{*} $p = 0.641$. ^{**} $p < 0.05$ by Chi-square trend test for analysis.

Table 2. Distribution of stool samples showing *Stx* positive by polymerase chain reaction compared with age

Age (yrs)	Farmer		Slaughterer	
	Positive* (%)	Negative (%)	Positive** (%)	Negative (%)
≤29	0.0	2.3	0.0	1.5
30–39	0.0	4.9	0.0	14.1
40–49	1.0	32.8	2.5	27.8
50–59	4.9	43.4	3.0	32.3
≥ 60	5.8	13.0	2.0	15.2
Total [#]	3.4	96.6	7.6	92.4

[#]Except the nonresponders. * $p = 0.01$. ** $p = 0.118$ by Chi-square trend test for analysis.

a questionnaire. The results in farmers indicated that the rate of *Stx* positivity at ‘over 30’ years of work was 5.6% and was the highest value. The percentage values of *Stx* positivity increase with years of work ($p = 0.052$; Table 3). In slaughterhouse workers, the rate of *Stx* positivity at ‘below 10’ years of work was 3.0% and was the highest value ($p = 0.235$).

3.4. Distribution of workfields of *Stx* positive workers in slaughterhouse workers

We divided the slaughterhouse workers into four categories: inspector, slaughterer, residual products handler, and livestock hygiene controller (Table 4).

The prevalence of *Stx* was found to be 6.3% (4/63) in residual products handlers, 8.9% (10/113) in slaughterers, and 4.5% (1/22) in livestock hygiene controllers. In this study, the prevalence of *Stx* in dairy farmers was 3.4% (21/621), which is lower than in the slaughterers but similar to that in the livestock hygiene controllers.

4. Discussion

Cattle are considered to be the main reservoir of STEC [10–12]. Recently, in a study of the prevalence

Table 3. Distribution of stool samples showing *Stx* positive by polymerase chain reaction compared with working years

Working year	Farmer		Slaughterer	
	Positive* (%)	Negative (%)	Positive** (%)	Negative (%)
≤10	1.4	11.7	3.0	39.4
10–19	1.2	27.5	1.0	15.7
20–29	4.8	44.3	1.5	12.1
≥30	5.6	10.8	0.5	26.8
Total [#]	3.5	96.5	6.1	93.9

[#]Except the nonresponders. * $p = 0.052$. ** $p = 0.235$ by Chi-square trend test for analysis.

Table 4. Distribution of stool samples showing *Stx* positive by polymerase chain reaction compared with slaughterhouse work fields

Work fields	Total	Positive	% *
Slaughterer	113	10	8.9
Livestock hygiene controller	22	1	4.5
Residual products handlers	63	4	6.3
Total	198	15	7.6

* $p = 0.046$ by Chi-square trend test for analysis.

study of STEC O157 in Swedish dairy herds, a rate of 8.9% STEC O157 was found [13,14]. In France, the prevalence of STEC was found to be 34.9% [15]. In India, the prevalence of STEC in cows and calves was found to be 18.9% and in dairy cattle was 32.4% [16]. Furthermore, in a study of Canadian dairy farm families, approximately 6% of the individuals were STEC carriers [17–19]. And in a follow-up study, the prevalence of *Stx* in dairy farmers was 5.3% (3/57) [20].

Among meat-processing company employees, the prevalence of STEC was as high as 9% [21,22]. Also, our previous survey results showed that 5.6% were STEC carriers in slaughterhouse workers [23]. Based on these reports, there is a distinct difference in the prevalence of STEC for dairy cattle by country, thus the altered trend in the prevalence of STEC for dairy farmers and slaughterhouse workers may have originated from different countries and different seasons. The majority of the reported human cases of STEC infection in the United Kingdom have been shown to occur in the warmer season [24]. This study was conducted in the colder season (Dairy farmer: November to April; slaughterhouse workers: October to April), thus the prevalence of STEC could have been influenced.

In this study, *Stx* PCR screening results of the dairy farmer was 3.4% and slaughterhouse workers was 7.6%. *Stx* PCR positive of slaughterhouse workers are more than two times higher than dairy farmers represented that slaughterhouse workers in EHEC are exposed to more than double that indicated. Among the three work fields in the slaughterhouse workers, the slaughterer showed the highest value and livestock hygiene controllers (3.4%) showed values near that of the dairy farmer. And, in dairy farmers, with STEC infection, age, working year are showed trend, as age and working years increased, the proportion of *stx*-positive farmers. Therefore, as increase as the contact with cattle, increase the risk of STEC infection in farmer and slaughterhouse workers.

All *stx* PCR positive workers had no symptoms of EHEC infection. The lack of disease associated with STEC infection in work field workers is interesting and may reflect protection associated with immunity induced by previous exposure. The role of asymptomatic human carriers as a source of contamination and, thus, the importance of personal hygiene measures in dairy farm

and in the meat-processing slaughterhouse should not be underestimated. We hope that there will be increased attention to the STEC infection from cattle to diminish the risks of infection and transmission. These results of the study show that slaughterhouse workers are at higher risk of STEC infection than farmers. In addition, slaughterhouse workers are a more potential source of food contamination and transmission.

Acknowledgements

This study was supported by the intramural grant (4835-300-210-13) of the Korea National Institute of Health.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.phrp.2011.11.045](https://doi.org/10.1016/j.phrp.2011.11.045)

References

- Adak GK, Wall PG, Smith HR, et al. PHLS begins a national case control study of *Escherichia coli* O157 infection in England. *Commun Dis Rep CDR Rev* 1996 Sep 13;6(10):144–6.
- Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998 Jan;11(1):142–201.
- Tozzi AE, Caprioli A, Minelli F, et al. Shiga toxin-producing *Escherichia coli* infections associated with hemolytic uremic syndrome, Italy, 1988–2000. *Emerg Infect Dis* 2003 Jan;9(1):106–8.
- Mellmann A, Bielaszewska M, Kock R, et al. Analysis of collection of hemolytic uremic syndrome-associated enterohemorrhagic *Escherichia coli*. *Emerg Infect Dis* 2008 Aug;14(8):1287–90.
- Melton-Celsa AR, O'Brien AD. Animal models for STEC-mediated disease. *Methods Mol Med* 2003;73:291–305.
- Zhang W, Bielaszewska M, Kuczius T, et al. Identification, characterization, and distribution of a Shiga toxin 1 gene variant (*stx1c*) in *Escherichia coli* strains isolated from humans. *J Clin Microbiol* 2002 Apr;40(4):1441–6.
- Zhang W, Bielaszewska M, Friedrich AW, et al. Transcriptional analysis of genes encoding Shiga toxin 2 and its variants in *Escherichia coli*. *Appl Environ Microbiol* 2005 Jan;71(1):558–61.
- Caprioli A, Morabito S, Brugere H, et al. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Vet Res* 2005 May-Jun;36(3):289–311.
- Korean Statistical Information Service. Livestock survey (In Korean) [cited 23 Jul 2010]. Available from: http://kosis.kr/nsportal/abroad/abroad_01List.jsp.
- Burnens AP, Frey A, Lior H, et al. Prevalence and clinical significance of verotoxin-producing *Escherichia coli* (VTEC) isolated from cattle in herds with and without calf diarrhoea. *Zentralbl Veterinarmed B* 1995 Jul;42(5):311–8.
- Hussein HS. Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. *J Anim Sci* 2007 Mar;85(Suppl. 13):E63–72.
- Roopnarine RR, Ammons D, Rampersad J, et al. Occurrence and characterization of verocytotoxigenic *Escherichia coli* (VTEC) strains from dairy farms in Trinidad. *Zoonoses Public Health* 2007 Mar;54(2):78–85.
- Eriksson E, Nerbrink E, Borch E, et al. Verocytotoxin-producing *Escherichia coli* O157:H7 in the Swedish pig population. *Vet Rec* 2003 Jun 7;152(23):712–7.
- Eriksson E, Aspan A, Gunnarsson A, et al. Prevalence of verotoxin-producing *Escherichia coli* (VTEC) O157 in Swedish dairy herds. *Epidemiol Infect* 2005 Apr;133(2):349–58.
- Fremaux B, Raynaud S, Beutin L, et al. Dissemination and persistence of Shiga toxin-producing *Escherichia coli* (STEC) strains on French dairy farms. *Vet Microbiol* 2006 Oct 31;117(2-4):180–91.
- Das SC, Khan A, Panja P, et al. Dairy farm investigation on Shiga toxin-producing *Escherichia coli* (STEC) in Kolkata, India with emphasis on molecular characterization. *Epidemiol Infect* 2005 Aug;133(4):617–26.
- Wilson JB, McEwen SA, Clarke RC, et al. Distribution and characteristics of verocytotoxigenic *Escherichia coli* isolated from Ontario dairy cattle. *Epidemiol Infect* 1992 Jun;108(3):423–39.
- Wilson JB, Clarke RC, Renwick SA, et al. Vero cytotoxigenic *Escherichia coli* infection in dairy farm families. *J Infect Dis* 1996 Nov;174(5):1021–7.
- Wilson J, Spika J, Clarke R, et al. Verocytotoxigenic *Escherichia coli* infection in dairy farm families. *Can Commun Dis Rep* 1998 Feb 1;24(3):17–20.
- Rahn K, Renwick SA, Johnson RP, et al. Follow-up study of verocytotoxigenic *Escherichia coli* infection in dairy farm families. *J Infect Dis* 1998 Apr;177(4):1139–40.
- Stephan R, Untermann F. Virulence factors and phenotypical traits of verotoxin-producing *Escherichia coli* strains isolated from asymptomatic human carriers. *J Clin Microbiol* 1999 May;37(5):1570–2.
- Stephan R, Ragetti S, Untermann F. Prevalence and characteristics of verotoxin-producing *Escherichia coli* (VTEC) in stool samples from asymptomatic human carriers working in the meat processing industry in Switzerland. *J Appl Microbiol* 2000 Feb;88(2):335–41.
- Hong S, Oh KH, Cho SH, et al. Asymptomatic healthy slaughterhouse workers in South Korea carrying Shiga toxin producing *Escherichia coli*. *FEMS Immunol Med Microbiol* 2009 Jun;56(1):41–7.
- Money P, Kelly AF, Gould SW, et al. Cattle, weather and water: mapping *Escherichia coli* O157:H7 infections in humans in England and Scotland. *Environ Microbiol* 2010 Oct;12(10):2633–44.