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# Shiga Toxin Subtypes Associated with Shiga Toxin–Producing *Escherichia coli* Strains Isolated from Red Deer, Roe Deer, Chamois, and Ibex

Eveline Hofer, Nicole Cernela, and Roger Stephan

## Abstract

A total of 52 Shiga toxin–producing *Escherichia coli* (STEC) strains, isolated from fecal samples of six ibex, 12 chamois, 15 roe deer, and 19 red deer were further characterized by subtyping the *stx* genes, examining strains for the top nine serogroups and testing for the presence of *eae* and *ehx*A. Eleven of the 52 strains belonged to one of the top nine STEC O groups (O26, O45, O91, O103, O111, O113, O121, O145, and O157). Eight STEC strains were of serogroup O145, two strains of serogroup O113, and one strain of serogroup O157. None of the strains harbored *stx*2a, *stx*2e, or *stx*2f. *Stx*2b (24 strains) and *stx*1c (21 strains) were the most frequently detected *stx* subtypes, occurring alone or in combination with another *stx* subtype. Eight strains harbored *stx*2g, five strains *stx*2d, three strains *stx*1a, two strains *stx*2c, and one strain *stx*1d. *Stx*2g and *stx*1d were detected in strains not harboring any other *stx* subtype. The *eae* and *ehx*A genes were detected in two and 24 STEC strains, respectively. Considering both, the serogroups and the virulence factors, the majority of the STEC strains isolated from red deer, roe deer, chamois, and ibex do not show the typical patterns of highly pathogenic STEC strains. To assess the potential pathogenicity of STEC for humans, strain isolation and characterization is therefore of central importance.

## Introduction

Shiga toxin (Stx)–producing *Escherichia coli* (STEC) is among the most common causes of foodborne diseases (Anonymous, 2011a). This organism is responsible for several human gastrointestinal illnesses, including nonbloody or bloody diarrhea. Especially in children, this disease may be affected by neurologic and renal complications, including hemolytic uremic syndrome (HUS). Most outbreaks and sporadic cases of bloody diarrhea and HUS have been attributed to strains of STEC serotype O157:H7. However, in Europe the role of non-O157 STEC strains (e.g., O26:H11/H–, O91:H21/H, O103:H2, O111:H–, O113:H21, O121:H19, O128:H2/H–, and O145:H28/H–) as a cause of HUS, bloody diarrhea, and other gastrointestinal illnesses is being increasingly recognized (Anonymous, 2011a).

The common feature and main virulence factor of STEC is production of Stx1 and/or Stx2 proteins. Human pathogenic STEC strains often may also harbor other virulence factors such as intimin (*eae*), a protein essential for the intimate attachment and the formation of attaching and effacing lesions on gastrointestinal epithelial cells, and EHEC-Enterohemolysin (*ehx*A) (Paton and Paton, 1998).

STEC have been isolated from feces of a variety of healthy domestic and wild animals, but domestic ruminants, especially cattle, are regarded as the principal reservoir of STEC for human infection (Karmali *et al.*, 2010). Nevertheless, wild ruminants may be a potential reservoir and therefore a source for human infections (Asakura *et al.*, 1998; Sanchez *et al.*, 2009; Bardiau *et al.*, 2010; Kistler *et al.*, 2011), and deer meat has been implicated in the transmission of STEC to humans (Keene *et al.*, 1997; Rabatsky-Ehr *et al.*, 2002; Ahn *et al.*, 2009; Rounds *et al.*, 2012). Minimal characterization data are available so far for STEC strains from wild ruminants and also for *stx* subtypes and virulence factors of non-O157 STEC strains from wild ruminants. Such data, however, are necessary to gain insight into the relationship of these strains and strains isolated from patients. In this study, we characterized strains isolated from red deer, roe deer, chamois, and ibex by (i) subtyping the *stx* genes, (ii) examining strains for the top nine serogroups (O26, O45, O91, O103, O111, O113, O121, O145, O157), and (iii) testing strains for the presence of *eae* and *ehx*A genes.

## Methods

### *Sampling and STEC detection*

Hunters collected fecal samples during the hunting season in autumn 2011 in the field, immediately after evisceration. For each sampled animal, sex, age, and location of hunting

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were recorded. After opening of the large intestine, fecal matter was collected from the colon, placed into sterile tubes, and stored frozen. In total, 239 faecal samples were obtained from three cantons in Switzerland (Graubünden, St. Gallen, Zug). Thereby, 84 samples originated from red deer (*Cervus elaphus*), 64 from roe deer (*Capreolus capreolus*), 64 from chamois (*Rupicapra rupicapra*), and 27 from ibex (*Capra ibex*), respectively. From each fecal sample about 1 g was enriched in 10 mL modified tryptic soy broth (mTSB, CMO989; Oxoid AG, Pratteln, Switzerland) supplemented with 16 mg/L novobiocin (novobiocin sodium; Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) for 18–24 h at 37°C. From 50 $\mu$L of the enrichment broth, a lysate was made (lysis tube; Pall GeneDisc Technologies, Bruz, France), and real-time polymerase chain reaction (PCR) for *stx* was performed using the commercially available GeneDisc system (Pall GeneDisc Technologies, Bruz, France), following manufacturer's instructions.

### *Isolation of STEC strains*

One loopful from the enrichment broth of each *stx*-positive sample was plated onto sheep blood agar (Difco™ Columbia Blood Agar Base EH, Becton Dickinson AG, Allschwil, Switzerland; 5% sheep blood SB055, Oxoid AG) and incubated for 18–24 h at 37°C. Thereafter, five colonies were picked, subcultivated on sheep blood agar for 18–24 h at 37°C, and tested with the LightCycler 2.0 (Roche Diagnostics AG, Rotkreuz, Switzerland) for the presence of *stx*1 and *stx*2 group genes by using the primers and probes described by Perelle *et al.* (2004).

### *Further characterization of STEC strains*

From each fecal sample with *stx*-positive isolates, one isolate was selected for further strain characterization. The strains were tested for the top nine serogroups (O26, O45, O91, O103, O111, O113, O121, O145, O157) by real-time PCR using GeneDisc system (Pall GeneDisc Technologies). Moreover, STEC strains were examined for the presence of *eae* and *ehxA* genes (Schmidt *et al.*, 1995; Møller Nielsen and Thorup Andersen, 2003). Shiga toxin genes were subtyped by PCR according to a standard procedure proposed by the World Health Organization Collaborating Center for Reference and Research on *Escherichia* and *Klebsiella* (Anonymous, 2011b). Strains were thereby tested for *stx*1a, *stx*1c, and *stx*1d if *stx*1-positive and for *stx*2a, *stx*2b, *stx*2c, *stx*2d, *stx*2e, *stx*2f, and *stx*2g if *stx*2-positive in the *stx* group specific PCR, respectively.

## Results

Of the 239 sampled animals, 103 (45%) tested *stx* positive in the screening PCR. By picking five colonies from each *stx*-positive sample, 52 STEC strains from 52 different animals

Table 1. Origin and Characteristics of Shiga Toxin–Producing *Escherichia coli* (STEC) Strains Isolated from Hunted Wild Ruminants

| *Number of strains* | *Origin* | *Serogroup* | stx*1-subtype* | stx*2-subtype* | eae | ehxA |
|---|---|---|---|---|---|---|
| 3 | Chamois | Others[a] | *stx*1c | — | − | + |
| 1 | Chamois | Others[a] | *stx*1c | — | − | − |
| 1 | Chamois | O145 | — | *stx*2b | − | − |
| 1 | Chamois | Others[a] | — | *stx*2b | − | + |
| 4 | Chamois | Other[a] | *stx*1c | *stx*2b | − | + |
| 2 | Chamois | O145 | *stx*1c | *stx*2b | − | − |
| 2 | Ibex | Others[a] | *stx*1c | — | − | + |
| 1 | Ibex | O113 | *stx*1c | *stx*2b | − | + |
| 1 | Ibex | O145 | — | *stx*2b | − | + |
| 1 | Ibex | Others[a] | *stx*1a | *stx*2b | − | − |
| 1 | Ibex | Other[a] | *stx*1c | *stx*2b | − | + |
| 1 | Red deer | Others[a] | *stx*1a | — | + | + |
| 1 | Red deer | O113 | *stx*1c | — | − | − |
| 1 | Red deer | Others[a] | *stx*1c | — | − | − |
| 3 | Red deer | O145 | — | *stx*2b | − | − |
| 2 | Red deer | Others[a] | — | *stx*2b | − | + |
| 2 | Red deer | Others[a] | — | *stx*2b | − | − |
| 1 | Red deer | O157 | — | *stx*2c | + | + |
| 1 | Red deer | Others[a] | — | *stx*2c, *stx*2d | − | + |
| 3 | Red deer | Others[a] | — | *stx*2d | − | − |
| 2 | Red deer | Others[a] | — | *stx*2g | − | − |
| 2 | Red deer | Other[a] | *stx*1c | *stx*2b | − | + |
| 1 | Roe deer | Others[a] | *stx*1c | — | − | + |
| 2 | Roe deer | Others[a] | *stx*1c | — | − | − |
| 2 | Roe deer | Others[a] | *stx*1d | — | − | − |
| 1 | Roe deer | O145 | — | *stx*2b | − | + |
| 2 | Roe deer | Others[a] | — | *stx*2b | − | − |
| 5 | Roe deer | Others[a] | — | *stx*2g | − | − |
| 1 | Roe deer | Others[a] | — | *stx*2g | − | + |
| 1 | Roe deer | Others[a] | *stx*1a | *stx*2d | − | + |

[a]Non O26, O45, O91, O103, O111, O113, O121, O145, and O157.

were isolated. Six of the strains originated from ibex, 12 from chamois, 15 from roe deer, and 19 from red deer, respectively. The characterization data of the strains for serogroups, *stx* subtypes, and the presence of *eae* and *ehxA* are summarized in Table 1. Eleven of the 52 isolated strains belonged to one of the top nine serogroups. Eight STEC strains were of serogroup O145, two strains of serogroup O113, and one strain of serogroup O157. None of the strains harbored *stx*2a, *stx*2e, and *stx*2f. *Stx*2b (24 strains) and *stx*1c (21 strains) were the most frequently detected *stx* subtypes, occurring alone or in combination with another *stx* subtype. Eight strains harbored *stx2*g, five strains *stx*2d, three strains *stx*1a, two strains *stx*2c, and one strain *stx*1d. *Stx*2g and *stx*1d were detected in strains not harboring any other *stx* subtype. The gene encoding for the outer membrane protein intimin was detected in two STEC strains from red deer. One of these strains was of serogroup O157. Twenty-four STEC strains (46%) tested positive for *ehxA*. There was no association between *stx*-subtype, the presence of *ehxA,* or origin of the strains.

## Discussion

STEC strains pathogenic for humans tend to feature Stx2 and other virulence traits such as the adhesion factor intimin (Friedrich *et al.*, 2002; Brooks *et al.*, 2005). In STEC strains characterized in this study, *stx*2 was the predominant *stx* gene identified, a result which is in agreement with previous studies on STEC isolates from wild ruminants (Sanchez *et al.*, 2009; Bardiau *et al.*, 2010; Kistler *et al.*, 2011) or isolates from game meat (Miko *et al.*, 2009). With respect to the *stx* subtypes (according to the established new Stx nomenclature) (Persson *et al.*, 2007), *stx*2b was the predominant variant among the STEC isolates in the present study. Subtype *stx*2b has also frequently been reported in STEC strains from deer meat (Miko *et al.*, 2009), and this variant most likely does not cause severe human diseases, since it is mainly found in strains isolated from healthy human carriers (Stephan and Hoelzle, 2000). Eight strains harbored *stx*2g, a *stx* variant which was originally described in a bovine fecal sample and which seems to have a minor role in human infections (Prager *et al.*, 2011). Moreover, *stx*1c-harboring strains, which were frequently found in the STEC from wild game, are associated with asymptomatic human carriage or mild disease (Friedrich *et al.*, 2003). Nevertheless, two of the isolates (one strain belonging to the O157 serogroup and one strain not belonging to the top nine serogroups) in the present study carried subtype *stx*2c, which has been associated with high virulence, and strains producing this *stx* subtype have been isolated from patients with hemolytic colitis and HUS (Friedrich *et al.*, 2002; Persson *et al.*, 2007; Käppeli *et al.*, 2011). Moreover, four other strains, isolated from three red deer and one roe deer, harbored *stx2*d. The Stx2d type also belongs to the group of Shiga toxin genes in the Stx2acd group (*stx*2a, *stx*2c, *stx*2d), which are genetically closely related and are reported to be associated with HUS in patients (Friedrich *et al.*, 2002; Persson *et al.*, 2007). The gene encoding for outer membrane protein intimin (*eae*) was detected only in two (one strain belonging to the O157 serogroup and one strain not belonging to the top nine serogroups) of the 52 STEC strains. In contrast the *ehx*A gene was found in high prevalence (46%). These results are in agreement with previous studies on STEC isolates from wild ruminants (Gilbreath *et al.*, 2009; Sánchez *et al.*, 2009; Bardiau *et al.*, 2010).

In summary, STEC can be detected in high frequency (45%) in fecal samples of wild ruminants. Considering both, the serogroups and the virulence factors, the majority of the STEC strains isolated from red deer, roe deer, chamois, and ibex do not show the typical patterns of highly pathogenic STEC strains. Nevertheless, highly pathogenic strains can be found. Therefore, to assess the potential pathogenicity of STEC strains from wild ruminants for humans, strain isolation and characterization are therefore of central importance.

## Disclosure Statement

No competing financial interests exist.

## References


Anonymous. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA J 2011a;9:2090.

Anonymous. WHO Collaborating Center for Reference and Research on *Escherichia* and *Klebsiella*. Identification of three *vtx*1 and seven *vtx*2 subtypes of Verocytotoxin encoding genes of *Escherichia coli* by conventional PCR amplification. 2011b. Available at: http://www.ssi.dk/English/PublicHealth/National%20Reference%20Laboratories/WHO%20Collaborating%20Centre%20for%20Reference%20and%20Research%20on%20Escherichia%20and%20Klebsiella.aspx, accessed December 12, 2011.

Ahn CK, Russo AJ, Howell KR, Holt NJ, Sellenriek PL, Rothbaum RJ, Beck AM, Luebbering LJ, Tarr PI. Deer sausage: A newly identified vehicle of transmission of *Escherichia coli* O157:H7. J Pediatr 2009;155:587–589.

Asakura H, Makino S, Shirahata T, Tsukamoto T, Kurazono H, Ikeda T, Takeshi K. Detection and genetical characterization of Shiga toxin–producing *Escherichia coli* from wild deer. Microbiol Immunol 1998;42:815–822.

Bardiau M, Grégoire F, Muylaert A, Nahayo A, Duprez J-N, Mainil J, Linden A. Enteropathogenic (EPEC), enterohaemorrhagic (EHEC) and verotoxigenic (VTEC) *Escherichia coli* in wild cervids. J Appl Microbiol 2010;109:2214–2222.

Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA. Non-O157 Shiga toxin–producing *Escherichia coli* infections in the United States, 1983–2002. J Infect Dis 2005;192:1422–1429.

Friedrich AW, Bielaszewska, Zhang W, Pulz M, Kuczius T, Ammon A, Karch H. *Escherichia coli* harboring Shiga toxin 2 gene variants: Frequency and association with clinical symptoms. J Infect Dis 2002;185:74–84.

Friedrich AW, Borell J, Bielaszewska M, Fruth A, Tschäpe H, Karch H. Shiga toxin 1c-producing *Escherichia coli* strains: Phenotypic and genetic characterization and association with human disease. J Clin Microbiol 2003;41:2448–2453.

Gilbreath JJ, Shields MS, Smith RL, Farrel LD, Sheridan PP, Spiegel KM. Shiga toxins, and the genes encoding for them, in fecal samples from native Idaho ungulates. Appl Environ Microbiol 2009;75:862–865.

Käppeli, U, Hächler H, Giezendanner N, Cheasty T, Stephan R. Shiga toxin–producing *Escherichia coli* O157 associated with human infections in Switzerland: 2000–2009. Epidemiol Infect 2011;139:1097–1104.

Karmali MA, Gannon V, Sargeant JM. Verocytotoxin-producing *Escherichia coli* (VTEC). Vet Microbiol 2010;140:360–370.

Keene WE, Sazie E, Kok J, Rice DH, Hancock DD, Balan VK, Zhao T, Doyle MP. An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from meat deer meat. JAMA 1997;277:1229–1231.

Kistler WM, Mulugeta S, and Mauro SA. Detection of $stx_1$ and $stx_2$ genes in Pennsylvanian white-tailed deer. Toxins 2011;3:640–646.

Miko A, Pries K, Haby S, Steege K, Albrecht N, Krause G, Beutin L. Assessment of Shiga toxin–producing *Escherichia coli* isolates from wildlife meat as potential pathogens for humans. Appl Environ Microbiol 2009;75:6462–6470.

Møller Nielsen E, Thorup Andersen M. Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5′ nuclease PCR assay. J Clin Microbiol 2003;41: 2884–2893.

Paton JC, Paton AW. Pathogenesis and diagnosis of Shiga toxin–producing *E. coli* infections. Clin Microbiol Rev 1998;11:450–479.

Perelle S, Dilasser F, Grout J, Fach P. Detection by 5′-nuclease PCR of Shiga-toxin–producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases. Mol Cell Probes 2004;18:185–192.

Persson S, Olsen KE, Ethelberg S, Scheutz F. Subtyping method for *Escherichia coli* shiga toxin (verocytotoxin) 2 variants and correlations to clinical manifestations. J Clin Microbiol 2007;45:2020–2024.

Prager R, Fruth A, Busch U, Tietze E. Comparative analysis of virulence genes, genetic diversity, and phylogeny of Shiga toxin 2g and heat-stable enterotoxin STLa encoding *Escherichia coli* isolates from humans, animals and environmental sources. Int J Med Microbiol 2011;301:181–191.

Rabatsky-Ehr R, Dingman D, Marcus R, Howard R, Kinney A, Mshar P. Deer meat as the potential source for a sporadic case of *Escherichia coli* O157:H7 infection, Connecticut. Emerg Infect Dis 2002;8:525–527.

Rounds JM, Rigdon CE, Muhl LJ, Forstner M, Danzeisen GT, Koziol BS, Taylor C, Shaw B T, Short GL, Smith KE. Non-O157 Shiga toxin–producing *Escherichia coli* associated with venison. Emerg Infect Dis 2012;18:279–282.

Sánchez S, García-Sánchez A, Martínez R, Blanco J, Blanco J E, Blanco M, Dahbi G, Mora A, Hermoso de Mendoza J, Alonso JM, Rey J. Detection and characterization of Shiga toxin–producing *Escherichia coli* other than *Escherichia coli* O157:H7 in wild ruminants. Vet J 2009;180:384–388.

Schmidt H, Beutin L, Karch H. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect Immun 1995;63:1055–1061.

Stephan R, Hoelzle LE. Characterization of verotoxin type 2 variant B-subunit in *Escherichia coli* strains from asymptomatic human carriers by PCR-RFLP. Lett Appl Microbiol 2000; 31:139–142.

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