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# Shiga toxin-producing *Escherichia coli* (STEC) shedding in a wild roe deer population

E. Frank[a,*], R. Bonke[a], N. Drees[a], M. Heurich[b], E. Märtlbauer[c], M. Gareis[a]

[a] Chair of Food Safety, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich, Schönleutnerstraße 8, D-85764 Oberschleißheim, Germany
[b] Department of Research and Documentation, Bavarian Forest National Park, Freyunger Straße 2, D-94481 Grafenau, Germany
[c] Chair of Hygiene and Technology of Milk, Faculty of Veterinary Medicine, Ludwig-Maximilians-Universität Munich, Schönleutnerstraße 8, D-85764 Oberschleißheim, Germany

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ABSTRACT

Worldwide infections by Shiga toxin-producing *Escherichia coli* (STEC) in humans have been reported after consumption of mainly beef, but also deer meat. Not only the consumption of contaminated deer meat represents a risk, but also the transmission of STEC between deer and domestic animals should be considered. Within the framework of a telemetry study of roe deer (*Capreolus capreolus*) the aim was to analyse the occurrence of STEC. Due to the chance to sample some animals several times it was possible to obtain data on the repeated shedding of STEC in roe deer. In total 124 faeces or rectal swabs of 77 live trapped roe deer were collected. The isolates obtained were characterized for *stx* subtypes, different virulence genes, the so-called top-five serogroups, phylogenetic groups, PFGE-types and antimicrobial susceptibilities.

The majority of roe deer were *stx*-positive whenever sampled. Twenty-eight animals were sampled more than once and were used to examine the duration of shedding STEC. The time interval of 6 persistently *stx*-negative tested animals was between 6 and 440d (median 49d, interquartile range (IQR) 17-258d). Ten animals excreted undistinguishable STEC strains in intervals between 4 and 778d (median 42d, IQR 22-79d).

Most of the isolates were *stx2b*-positive, *eae*-negative and frequently *ehlyA*-positive. None of the isolates belonged to serogroup O26, O103, O111, O145 and O157, respectively. All isolates were sensitive to the antimicrobial substances tested. Although the duration of each shedding event could not be determined the results indicate long-term excretion of STEC in roe deer. This is an important consideration for the observance of good hygiene practice while field dressing of deer and preparing deer meat.

## 1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are among the relevant foodborne diseases with zoonotic potential. In 2017, 6073 people became infected by STEC in the EU (EFSA and ECDC, 2018). Humans suffering from an STEC infection can show symptoms ranging from mild diarrhoea to haemolytic-uremic syndrome (HUS). The human pathogenicity of STEC depends, among other parameters, on the toxin type, virulence genes and the serotype. Shiga toxin genes are subdivided into *stx1a, stx1c, stx1d* and *stx2a-stx2 g* (Scheutz et al., 2012). Patients with HUS are frequently tested positive for STEC with *stx2a* and *stx2d* (Scheutz et al., 2012). Important virulence genes are *eae* and *e-hlyA*, which are often isolated in STEC strains from patients suffering from haemorrhagic colitis (HC) or HUS (Beutin and Fach, 2014). The STEC-autoagglutinating-adhesin gene (*saa*) exists more probable in *eae*-negative STEC strains (Ferdous et al., 2016). Although several STEC serogroups were detected in patients, the so-called top-five STEC serogroups, which are often linked to human diseases, were O26, O103, O111, O145 and O157 (EFSA and ECDC, 2018).

*Escherichia (E.) coli* can be divided into 8 phylogenetic groups (A, B1, B2, C, D, E, F, cryptic clade I) providing an indication of the pathogenicity of the strains. *E. coli* causing extra-intestinal diseases are mostly belonging to the phylogenetic groups B2 and D (Clermont et al., 2000, 2013). STEC from calves were mostly part of group B1 but also other groups were described (A, B2, C, E, unknown) (Coura et al., 2017).

The transmission of STEC strains is possible through direct contact between humans, between animals or between animals and humans, but occurs primarily through consumption of contaminated food products (Henderson, 2008; Beutin and Fach, 2014). Ruminants, especially cattle, are the main reservoir of this pathogen (Lim et al., 2010). The average duration of shedding of serotype O157:H7 of cattle is about 30

* Corresponding author.
*E-mail address:* Sekretariat@ls.vetmed.uni-muenchen.de (E. Frank).

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days, with variable time frames of a few days to a whole year (Henderson, 2008). Similar time intervals are described for children with diseases caused by O157 and other serogroups as well (Dabke et al., 2014; Matussek et al., 2015).

Some studies have dealt with the occurrence of STEC in wild ruminants in Europe (Kemper et al., 2006; Lehmann et al., 2006; Sánchez et al., 2009; Bardiau et al., 2010; Eggert et al., 2012; Mora et al., 2012; Obwegeser et al., 2012; Laaksonen et al., 2017). However, none of these studies paid attention to the duration of shedding STEC. Fischer et al. (2001) were able to examine the duration of shedding for serotype O157:H7 at inoculated white-tailed deer (Odocoileus virginus) in Georgia, USA, over a period of 26 days. Singh et al. (2015) observed the shedding of STEC in 12 white-tailed deer over a period of 3 month during a transmission study of STEC between cattle and white-tailed deer in Michigan, USA. To the best of our knowledge no further studies, neither on inoculated nor on naturally colonized deer, exist.

Therefore the samples available for this study provided the opportunity to get first data on the repeated shedding of STEC in healthy wild roe deer. Additionally, obtained isolates were characterized for *stx* subtypes, different virulence genes, so-called top-five serogroups, phylogenetic groups and PFGE-types. Although antimicrobial treatment of STEC infections is commonly not recommend, the incidence of resistant STEC with varying prevalence has been reported (Mora et al., 2005). For this reason, the antimicrobial sensibility of STEC isolates from deer to 14 different antimicrobial effective substances was tested.

## 2. Methods

### 2.1. Samples

Within the framework of a telemetry study of roe deer (Ciuti, 2018), 124 samples of faeces and rectal swabs of live roe deer were collected in the Bavarian Forest National Park and adjacent shoot territories. A description of the research area and the wildlife management is available in Möst et al. (2015). The wild living roe deer was lured into live catch traps for single animals by feeding. Which animal or the number of times an animal was captured could not be influenced.

The first 23 faeces samples were taken in February, March and August 2010 from the ground of a live catch trap for single animals. From November 2010 until March 2011, 46 rectal swabs, from November 2011 until March 2012, 47 rectal swabs and from January until March 2013, 8 rectal swabs were taken from the trapped animals.

Due to organisational reasons the faeces samples from the first period have been frozen at - 20 °C, while the swabs were placed in 10 ml buffered peptone water (3 M™ Swab-Sampler with Buffered Peptone Water Broth; 3 M, Neuss, Germany) and refrigerated until analysis.

### 2.2. Detection of the stx genes

The faeces samples (1 g) were enriched in 90 ml buffered peptone water (BPW; Merck, Darmstadt, Germany) for 20 h at 37 °C. The rectal swabs with buffered peptone water were enriched for 20 h at 37 °C. Prior to incubation one loop of the enrichment broth was directly streaked onto sorbitol-MacConkey agar plate (SMAC; Merck) and incubated for 24 h at 41.5 °C. After overnight enrichment, DNA extraction was performed directly from 100 µl of rectal swab broth with InstaGene™Matrix (Bio-Rad, Munich, Germany) according to the manufacturer's protocol. For the faecal sample analysis one loop of the overnight enrichment was streaked onto SMAC agar plate and incubated for 24 h at 41.5 °C. Afterwards the DNA was extracted from the first streaking area of each of these SMAC agar plate. Therefore, the material was suspended in 100 µl molecular biology grade water and the DNA was extracted by heating (99 °C, 10 min). After centrifugation, the supernatant was used as DNA template.

The multiplex real-time PCR for the detection of *stx*1 and *stx*2 with an inhibition control was carried out in 20 µl volumes containing 10 µl SsoFast™EvaGreen® Supermix (BioRad, Munich, Germany), 200 nM of each primer pair (*stx1* forward + reverse, *stx2* forward + reverse (Sharma and Dean-Nystrom, 2003), IPC fw + re (Messelhäusser et al., 2007)), 1 µl pUC19 (1 fg/µl) and 1 µl of the DNA template. All real-time PCRs were performed in a CFX Cycler (Bio-Rad). Thermal cycling consisted of denaturation (98 °C for 5 s), annealing and extension (58 °C for 15 s), performed in 40 cycle steps. Melting curve analysis ranged from 65 °C to 95 °C with 0.5 °C intervals.

### 2.3. Isolation of STEC

The corresponding rectal swabs enrichment cultures of *stx*-positive samples were diluted 1:1 000 000 and faecal enrichment cultures of *stx*-positive samples were diluted 1:100 000. A total of 100 µl of the dilution was plated onto SMAC agar and incubated for 24 h at 41.5 °C. From each directly streaked out agar plate and after overnight enrichment and dilution plated agar plate, 2–10 colonies (sorbitol-positive and -negative) were randomly chosen and analysed for *stx1* and *stx2* by multiplex real-time PCR. The PCR assays were carried out in 20 µl volumes containing 10 µl SsoFast™EvaGreen® Supermix, 200 nM of each primer pair (*stx1* forward + reverse, *stx2* forward + reverse (Sharma and Dean-Nystrom, 2003)), 5 µl molecular biology grade water and 1 µl of the DNA template. Thermal cycling was performed like described above with an annealing and extension of 56 °C for 15 s. Colonies which were *stx*-positive were further subcultured.

*Stx*-positive isolates were confirmed as *E. coli* by API® 20E (bioMérieux, Nürtingen, Germany). Pure cultures were investigated for *stx1a, stx1c, stx1d, stx2a, eae, e-hlyA* and *saa* by real-time and for *stx2b, stx2c, stx2d* by conventional PCR. The real-time PCR assays were carried out in 20 µl volumes containing 10 µl SsoFast™EvaGreen® Supermix, 200 nM of the respective primer pair (stx1a-F1 + R2, stx1c-F1 + R1, stx1d-F1 + R2, stx2a-F2 + R2 + R3 (Scheutz et al., 2012), eae-F2 + R, ehec-F + R, Saa-F + R (Nielsen and Andersen, 2003)), 7 µl (*stx2a*: 3 µl) molecular biology grade water and 1 µl of the DNA template. Thermal cycling consisted of denaturation (98 °C for 10 s (*eae, e-hlyA* and *saa*: 5 s)), annealing and extension (66 °C for 40 s (*eae, e-hlyA* and *saa*: 56 °C for 15 s)), performed in 40 cycle steps. Melting curve analysis ranged from 65 °C to 95 °C with 0.5 °C intervals. The conventional PCR assays were carried out in 25 µl volumes containing 0,25 µl Go Taq® G2 Flexi DNA Polymerase (Promega, Mannheim, Germany), 200 nM of the respective primer pair (stx2b-F1 + R1, stx2c-F1 + R2, stx2d-F1 + R1 + R2 (Scheutz et al., 2012)), 2,5 µl dNTP´s (2 mM, Promega), 2 µl $MgCl_2$ (25 nM, Promega), 5 µl Green Go Taq® Flexi Buffer and 1 µl of template from pure *stx*-positive cultures. The PCR was performed in an iCycler (Bio-Rad). Thermal cycling consisted of denaturation (95 °C for 30 s (*stx 2b*: 15 s)), annealing and extension (60 °C for 30 s (*stx 2b*: 15 s) and 72 °C for 30 s (*stx 2b*:10 s)), performed in 35 (*stx 2b*: 30) cycle steps. Agarose Gel was 1% (*stx 2b*: 2%).

### 2.4. Serotyping

The analysis of the isolates for the serogroups O26, O103, O111, O145 and O157 was performed using real-time PCR assays in 20 µl volumes containing 10 µl SsoFast™EvaGreen® Supermix, 200 nM of the respective primer pair (*wzx* (O26) f + r, *wzx* (O103) f + r, *wbdI* (O111) f + r, *ihp1* (O145) f + r, *rfbE* (O157) f + r (European Food Safety Authority (EFSA, 2009)), 7 µl molecular biology grade water and 1 µl of template from pure *stx*-positive cultures. The PCR was performed in a CFX Cycler. Thermal cycling consisted of denaturation (98 °C for 5 s), annealing and extension (59 °C for 20 s) in 40 cycle steps. Melting curve analysis ranged from 65 °C to 95 °C with 0.5 °C intervals.

### 2.5. Antimicrobial susceptibility

The antimicrobial susceptibility testing of the isolates was performed using commercial broth microdilution tests (CMV3AGNF

Sensititre® (TREK Diagnostic Systems, East Grinstead, UK) or. VetMIC™GN-mo (SVA, Uppsala, Sweden)). The Tests were used according to the manufacturers' protocol and evaluated as described before (Drees, 2012). The phenotypic sensitivity of following 14 antimicrobial substances was examined: cefoxitin, azithromycin, chloramphenicol, tetracycline, ceftriaxone, amoxicillin / clavulanic acid 2:1 ratio, ciprofloxacin, gentamicin, nalidixic acid, ceftiofur, sulfisoxazole, trimethoprim / sulfamethoxazole, ampicillin and streptomycin or. ampicillin, ciprofloxacin, nalidixic acid, gentamicin, tetracycline, streptomycin, sulfamethoxazole, trimethoprim, florfenicol, colistin, kanamycin, chloramphenicol, cefotaxim and ceftazidim.

### 2.6. Phylogenetic groups

To affiliate the isolates to the phylogenetic group as described by Clermont et al. (2000) and Clermont et al. (2013), three real-time PCR approaches were performed for *chuA, yjaA, TSPE4.C2* and a conventional PCR for *arpA*. The real-time PCR assays were carried out in 20 µl volumes containing 10 µl SsoFast™EvaGreen® Supermix, 200 nM of the respective primer pair (ChuA.1 + 2, YjaA.1 + 2, TspE4C2.1 + 2 (Clermont et al., 2000)), 7 µl molecular biology grade water and 1 µl of template from pure *stx*-positive cultures. The PCR was performed in a CFX Cycler. Thermal cycling consisted of denaturation (98 °C for 5 s), annealing and extension (60 °C for 30 s), performed in 40 cycle steps. Melting curve analysis ranged from 65 °C to 95 °C with 0.5 °C intervals. The conventional PCR assays were carried out in 25 µl volumes containing 0.25 µl Go Taq® G2 Flexi DNA Polymerase, 200 nM of the primer pair AceK.f + ArpA1.r (Clermont et al., 2013), 2.5 µl dNTP´s (2 mM, Promega), 2 µl $MgCl_2$ (25 nM, Promega), 5 µl Green Go Taq® Flexi Buffer and 1 µl of template from pure *stx*-positive cultures. The PCR was performed like described above.

### 2.7. PFGE

The PFGE Analysis was performed as previously described (Eggert et al., 2012). The pulse time were ramped from 6.75 to 35.38 s over 21 h. The obtained data was analysed by BioNumerics Version 7.6.2 (Applied Maths, Sint-Martens-Latem, Belgium). Percentages of similarity between fingerprints were determined using the band-based Dice coefficient and a 5% band position tolerance. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) on a matrix resulting from comparison of PFGE-*Xba*I patterns was used for generating a dendrogram.

## 3. Results

### 3.1. Occurrence of STEC

In total, 124 samples were collected from 77 roe deer (Table 1). From 49 of these 77 animals, only one sample could be examined, because these animals got trapped only a single time. Out of the 28 roe deer captured more than once, 17 animals were captured two times and 11 animals more than twice (7 animals three times, 2 animals four times, 1 animal five times and 1 animal 7 times). In time intervals from 4 up to 778 days in total 75 samples were obtained (Table 2–4) for this subgroup. Six of these animals (15 samples) remained *stx*-negative from 6 up to 440 days (Table 4). Sixteen animals were *stx*-positive in every sample (intervals from 4 up to 778 days) (Table 2). Six animals were tested both *stx*-positively and *stx*-negatively. In 5 cases the first sample was *stx*-negative and the following samples were *stx*-positive and one of these animals was *stx*-negative in the last sample again. In one case, an animal (#69) was *stx2*-positve at first, became *stx*-negative in the second sample and then *stx1*-positive for two times (Table 3).

### 3.2. Isolation of STEC

Out of the 16 persistently *stx*-positive tested animals, 67 isolates could be obtained from 35 *stx*-positive samples. It was not possible to gather an isolate out of four *stx*-positive samples (Table 1). Out of the six animals with both *stx*-positive and *stx*-negative samples, 29 isolates could be obtained from 13 *stx*-positive samples. It was not possible to gather an isolate out of one *stx*-positive samples and seven samples were *stx*-negative. All 96 isolates were sorbitol-positive.

### 3.3. Virulence gene profile

Seventy-seven isolates were positive for the *stx2* gene and 19 of the 96 isolates were tested positive for *stx1* gene (animals #69, 82, 124, 187) (Table 2, 3). One *stx1*-positive isolate could be further subtyped to the two subtypes *stx1a* and *stx1c* (#82). The remaining 18 *stx1*-positive isolates could be further subtyped to *stx1c*. Subtyping of the *stx2*-positive isolates resulted in the detection of three different subtypes: *stx2b, stx2c* and *stx2d*. Of these, two isolates (#94) were *stx2c*- and *stx2d*-positive, while *stx2b* was detectable in the remaining 75 *stx2*-positive isolates. None of the isolates were tested positive for the subtypes *stx1d* and *stx2a*. The virulence gene *e-hlyA* was present in 43 isolates (44.8%). *Saa* was detected in six isolates (5.8%) from four animals (#69, 94, 124, 183). None of the isolates carried the virulence gene *eae*.

### 3.4. Analysis of the serogroup

None of the 96 isolates belongs to one of the analysed O-types (O26, O103, O111, O145 and O157).

### 3.5. Antimicrobial susceptibility

All 96 isolates were sensitive in the examination of antimicrobial susceptibilities.

### 3.6. Phylogenetic groups

All 96 isolates could be matched to the phylogenetic groups B1 or B2 (Table 2, 3). All *stx1c*-positive isolates were member of the phylogenetic group B1.

**Table 1**
Overview about single and repeatedly sampled animals and the obtained isolates.

| | | samples | | | *stx*-positive samples | | STEC isolates | | |
|---|---|---|---|---|---|---|---|---|---|
| | animals | total | *stx*-negative | *stx*-positive | without isolates | with isolates | total | *stx1*-positive | *stx2*-positive |
| total | 77 | 124 | 34 | 90 | 13 | 77 | 143 | 21 | 122 |
| single sampled | 49 | 49 | 12 | 37 | 7 | 30 | 47 | 2 | 45 |
| repeatedly sampled | 28 | 75 | 22 | 53 | 5 | 48 | 96 | 19 | 77 |
| persistently *stx*-negative sampled | 6 | 15 | 15 | 0 | – | – | – | – | – |
| persistently *stx*-positive sampled | 16 | 39 | 0 | 39 | 4 | 35 | 67 | 8 | 59 |
| *stx*-negatively and *stx*-positively sampled | 6 | 21 | 7 | 14 | 1 | 13 | 29 | 11 | 18 |

**Table 2**
Sample results of 16 persistently *stx*-positive tested roe deer.

| animal # | time interval [d] | result | isolate | *stx* subtype | virulence genes | phylogenetic group | PFGE-*Xba*I type |
|---|---|---|---|---|---|---|---|
| 1 | 0 | *stx2* | none | | | | |
| 1 | 160 | *stx2* | CCF61c.1ON | 2b | - | B2 | B |
| | | | CCF61c.2ON | 2b | - | B2 | C |
| 53 | 0 | *stx2* | CCF40.2P | 2b | - | B2 | S |
| 53 | 347 | *stx2* | CCRS47.1 D | 2b | *ehlyA* | B1 | O |
| | | | CCRS47.1 ON | 2b | *ehlyA* | B1 | O |
| 66 | 0 | *stx2* | CCRS32A.1 D | 2b | - | B2 | A |
| 66 | 778 | *stx2* | CCRS203.3 D | 2b | - | B2 | A |
| | | | CCRS203.1 ON | 2b | - | B2 | A |
| | | | CCRS203.2 ON | 2b | - | B2 | A |
| 94 | 0 | *stx2* | CCF43.1D | 2c, 2d | *ehlyA, saa* | B1 | N |
| | | | CCF43.2D | 2c, 2d | *ehlyA* | B1 | N |
| | | | CCF43.2P | 2b | *ehlyA* | B1 | G |
| | | | CCF43.2N | 2b | *ehlyA, saa* | B1 | G |
| 94 | 4 | *stx2* | CCF33.2D | 2b | *ehlyA* | B1 | G |
| 107 | 0 | *stx2* | CCF44.2N | 2b | *ehlyA* | B1 | H |
| 107 | 302 | *stx2* | none | | | | |
| 120 | 0 | *stx2* | none | | | | |
| 120 | 302 | *stx2* | CCRS7.1 D | 2b | - | B2 | B1° |
| 151 | 0 | *stx2* | none | | | | |
| 151 | 405 | *stx2* | CCRS66.2s.1ON | 2b | - | B2 | Q |
| 156 | 0 | *stx2* | CCRS19.1 D | 2b | - | B1 | F1° |
| | | | CCRS19.2 ON | 2b | - | B1 | F2° |
| 156 | 44 | *stx2* | CCRS36.1 D | 2b | - | B1 | F |
| | | | CCRS36.1 ON | 2b | - | B1 | F |
| 158 | 0 | *stx2* | CCRS21A.1 D | 2b | *ehlyA* | B2 | T2° |
| | | | CCRS21A.1 ON | 2b | *ehlyA* | B2 | T |
| 158 | 38 | *stx2* | CCRS40.1 D | 2b | *ehlyA* | B2 | T1° |
| | | | CCRS40.2 ON | 2b | *ehlyA* | B2 | T3° |
| 158 | 45 | *stx2* | CCRS55.1 D | 2b | - | B1 | F |
| | | | CCRS55.1 ON | 2b | *ehlyA* | B2 | T |
| 159 | 0 | *stx2* | CCRS9 D | 2b | - | B2 | B |
| | | | CCRS9.3 ON | 2b | - | B2 | B |
| 159 | 67 | *stx2* | CCRS48.1 D | 2b | *ehlyA* | B2 | B |
| | | | CCRS48.1 ON | 2b | - | B2 | B |
| 159 | 91 | *stx2* | CCRS46.2 D | 2b | - | B2 | B |
| | | | CCRS46.1 ON | 2b | - | B2 | B |

(*continued on next page*)

### 3.7. PFGE patterns

The 96 isolates yielded 31 different *Xba*I profiles. From the samples of four deer (#82, 107, 120, 151) only one isolate was obtained. Each of these isolates had PFGE-*Xba*I types which did not match with any other isolate. Isolates obtained of ten animals (#66, 69, 94, 108, 158, 159, 163, 168, 183, 187) had undistinguishable isolate bands at various times (Table 2, 4). Animal #69 first carried isolates of PFGE-*Xba*I type F (*stx*2) and after a negative sample several isolates with PFGE-*Xba*I type G or G1 (*stx1*) could be obtained. All *stx1c* positive isolates samples of the deer #69, 124, 187 had the PFGE-*Xba*I type G, G1 or G2 with undistinguishable bands either with one or two different bands.

**Table 2** (*continued*)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 163 | 0 | *stx2* | CCRS13 D | 2b | - | B2 | B |
| | | | CCRS13.3 ON | 2b | - | B2 | B |
| 163 | 23 | *stx2* | CCRS50.1 D | 2b | - | B2 | B |
| | | | CCRS50.2 ON | 2b | - | B2 | B |
| 163 | 63 | *stx2* | CCRS41.1 D | 2b | *ehlyA* | B2 | B |
| 183 | 0 | *stx2* | CCRS81.1ch.1eD | 2b | *saa* | B2 | P |
| | | | CCRS81.1e.1D | 2b | - | B2 | P |
| | | | CCRS81.1s.1D | 2b | - | B2 | P |
| | | | CCRS81.1e.1ON | 2b | - | B2 | P |
| | | | CCRS81.1s.1ON | 2b | - | B2 | P |
| 183 | 10 | *stx2* | CCRS74.1ch.1D | 2b | - | B2 | P |
| | | | CCRS74.1ch.1ON | 2b | - | B2 | P |
| 183 | 16 | *stx2* | CCRS61.1ch.1D | 2b | - | B2 | E |
| | | | CCRS61.2s.1ON | 2b | - | B2 | P |
| 183 | 29 | *stx2* | CCRS94.1chON | 2b | - | B2 | P |
| | | | CCRS94.1s ON | 2b | *saa* | B2 | P |
| 183 | 51 | *stx2* | CCRS99.1c D | 2b | - | B2 | P |
| | | | CCRS99.2s ON | 2b | - | B2 | E |
| 187 | 0 | *stx1* | CCRS86.1ch.1.1eD | 1c | *ehlyA* | B1 | M |
| | | | CCRS86.1eD | 1c | *ehlyA* | B1 | M |
| | | | CCRS86.1sD | 1c | *ehlyA* | B1 | M |
| | | | CCRS86.2e.1ON | 1c | *ehlyA* | B1 | M |
| | | | CCRS86.1s.1ON | 1c | *ehlyA* | B1 | M |
| 187 | 21 | *stx1* | CCRS92.2chD | 1c | *ehlyA* | B1 | M |
| 187 | 351 | *stx1* | CCRS216.1 D | 1c | *ehlyA* | B1 | M |
| | | | CCRS216.2 ON | 1c | *ehlyA* | B1 | M |
| 188 | 0 | *stx2* | CCRS65.1sD | 2b | - | B2 | B |
| 188 | 7 | *stx2* | CCRS82.1e.1D | 2b | - | B2 | R |
| 190 | 0 | *stx2* | CCRS60.1ch.1D | 2b | - | B2 | K1° |
| | | | CCRS60.1ch.2D | 2b | - | B2 | K1° |
| 190 | 412 | *stx2* | CCRS219.3 D | 2b | - | B2 | K |
| | | | CCRS219.7 ON | 2b | - | B2 | K |
| 206 | 0 | *stx2* | CCRS90.1chD | 2b | *ehlyA* | B2 | J |
| 206 | 16 | *stx2* | CCRS24.1chD | 2b | *ehlyA* | B2 | J1° |

° - one or two bands differs.

## 4. Discussion

This study tried to analyse the excretion time of STEC of randomly sampled wild roe deer. Twenty-eight animals were sampled more than one time and could, therefore, be used to give a first indication on the duration of shedding. It was shown that 10 animals excreted probably the same strain over a larger period of time (Table 4). These 10 *stx*-positive animals were tested at irregular intervals between 4 and 778 days (median 42d, IQR 22-79d). The as well irregular time interval between the six animals, which were always *stx*-negative, was between 6 and 440d (median 49d, IQR 17-258d).

Singh et al. (2015) studied STEC transmission between cattle and free-ranging white-tailed deer, by collecting 85 deer faeces from 73 animals in March (all STEC negative), 78 deer faeces from 74 animals in June (7 STEC positive) and 100 faecal grabs of cattle (26 STEC positive) in July from the same area. They recovered highly similar *stx1*-positive STEC isolates from several cattle and one deer and were able to show that the pathogen positive deer samples clustered spatially. Twelve white-tailed deer could be examined consecutively in March and June. In March all deer samples were STEC negative, while in June three were STEC positive. Based on these results they supported the theory of transmission across species and pointed out the possibility that the pathogens could persist in specific environments.

Also Fischer et al. (2001) examined the duration of shedding STEC in another but closely related deer species for 26 days. The excretion rate of STEC decreased drastically after 2 weeks and was only

**Table 3**
Sample results of six *stx*-positive and -negative tested roe deer.

| animal # | time interval [d] | result | isolate | *stx* subtype | virulence genes | phylogenetic group | PFGE-*Xba*I type |
|---|---|---|---|---|---|---|---|
| 67 | 0 | *negative* | - | | | | |
| 67 | 50 | *stx2* | CCRS11.1 D | 2b | *ehlyA* | B1 | L |
| | | | CCRS11.1.2 ON | 2b | *ehlyA* | B1 | L |
| 69 | 0 | *stx2* | CCRS39.1 D | 2b | - | B2 | D |
| | | | CCRS39.2 ON | 2b | - | B2 | D |
| 69 | 360 | *negative* | - | | | | |
| 69 | 412 | *stx1* | CCRS101.1c.1D | 1c | *ehlyA,saa* | B1 | M |
| | | | CCRS101.1s.1D | 1c | *ehlyA* | B1 | M |
| | | | CCRS101.1s ON2 | 1c | *ehlyA* | B1 | M1° |
| 69 | 747 | *stx1* | CCRS224.4 D | 1c | *ehlyA* | B1 | M |
| | | | CCRS224.1 ON | 1c | *ehlyA* | B1 | M |
| | | | CCRS224.2 ON | 1c | *ehlyA* | B1 | M |
| 82 | 0 | *negative* | - | | | | |
| 82 | 314 | *stx1* | CCRS18.2 ON | 1a, 1c | - | B1 | I |
| 108 | 0 | *negative* | - | | | | |
| 108 | 173 | *stx2* | none | | | | |
| 108 | 390 | *stx2* | CCRS54.1 ON | 2b | *ehlyA* | B2 | B |
| 108 | 710 | *stx2* | CCRS77.1ch.1sD | 2b | - | B2 | B |
| | | | CCRS77.1eD | 2b | - | B2 | B |
| | | | CCRS77.3s.1ON | 2b | - | B2 | B |
| 108 | 738 | *stx2* | CCRS22.1chD | 2b | *ehlyA* | B2 | B |
| 108 | 752 | *stx2* | CCRS98.1c D | 2b | - | B2 | B |
| | | | CCRS98.1s D | 2b | - | B2 | B |
| | | | CCRS98.1s ON | 2b | - | B2 | B |
| | | | CCRS98.2s ON | 2b | - | B2 | A1° |
| 108 | 766 | *stx2* | CCRS103.2c D | 2b | - | B2 | B |
| 124 | 0 | *negative* | - | | | | |
| 124 | 711 | *stx1* | CCRS67.1ch.1D | 1c | *ehlyA, saa* | B1 | M |
| | | | CCRS67.1s.1D | 1c | *ehlyA* | B1 | M2° |
| | | | CCRS67.1e.1D | 1c | *ehlyA* | B1 | M |
| | | | CCRS67.2s.1ON | 1c | *ehlyA* | B1 | M |
| 124 | 725 | *negative* | - | | | | |
| 168 | 0 | *negative* | - | | | | |
| 168 | 412 | *stx2* | CCRS91.1s.1D | 2b | *ehlyA* | B1 | L |
| | | | CCRS91.1e ON | 2b | *ehlyA* | B1 | L |
| | | | CCRS91.1s ON | 2b | *ehlyA* | B1 | L |
| 168 | 419 | *stx2* | CCRS27.1e ON | 2b | *ehlyA* | B1 | L |

° - one or two bands differs.

intermittently detectable afterwards. The strain could be detected up to day 24. These results, however, cannot directly be compared with the results of our study, because the white-tailed deer were artificially inoculated with STEC O157:H7 while captured and examined for 26 days.

Until now most information about the duration of shedding of STEC is available for human and cattle for O157:H7. According to the literature, duration of shedding STEC by cattle and calves is about 30d, but may also vary from a few days up to a year (Henderson, 2008). For example at Swedish children, the median *stx* shedding duration was 20 days (1-256d), although no difference could be determined between the *stx* subtypes (*stx1*, *stx2*, *stx1* + *stx2*) (Matussek et al., 2015). Dabke et al. (2014) observed a median duration of STEC shedding of 31 days at

**Table 4**
Time interval of 6 persistently *stx*-negative roe deer and of 10 roe deer with undistinguishable strains.

| animal # | time interval [d] | result | PFGE-*Xba*I type |
|---|---|---|---|
| 147 | 13 | *negative* | - |
| 147 | 26 | *negative* | - |
| 150 | 17 | *negative* | - |
| 160 | 440 | *negative* | - |
| 164 | 49 | *negative* | - |
| 164 | 69 | *negative* | - |
| 164 | 372 | *negative* | - |
| 181 | 258 | *negative* | - |
| 197 | 6 | *negative* | - |
| 66 | 778 | *stx2b* | A |
| 69 | 335 | *stx1c, ehlyA* | M |
| 94 | 4 | *stx2b, ehlyA* | G |
| 108 | 348 | *stx2b, ehlyA* | B |
| 108 | 42 | *stx2b* | B |
| 108 | 56 | *stx2b* | B |
| 158 | 45 | *stx2b, ehlyA* | T |
| 159 | 67 | *stx2b* | B |
| 159 | 91 | *stx2b* | B |
| 163 | 23 | *stx2b* | B |
| 168 | 7 | *stx2b, ehlyA* | L |
| 183 | 29 | *stx2b, saa* | P |
| 183 | 10 | *stx2b* | P |
| 183 | 16 | *stx2b* | P |
| 183 | 29 | *stx2b* | P |
| 183 | 51 | *stx2b* | P |
| 183 | 35 | *stx2b* | E |
| 187 | 21 | *stx1c, ehlyA* | M |
| 187 | 351 | *stx1c, ehlyA* | M |

children in England (IQR 17-41d). Both studies analysed STEC cases with different serotypes until the first negative sample was identified.

Unfortunately it was not possible to receive samples from the wild roe deer in regular intervals. Therefore it is possible, especially during the long sampling intervals, that animals were STEC negative and later re-infected. Considering this limitation, a median duration of shedding of 42 days was calculated. Likewise, the time frame varied from a few days up to several month with a maximum of 778d (Table 4).

In the study by Singh et al. (2015) 9 of 12 animals remained negative for an interval of 90 days. In this study it was shown that 6 roe deer remained *stx*-negative between 6–440 days even though it cannot be ensured, that animals excreted STEC in the meantime. For this, a more frequent and closer sampling interval would be necessary, which turns to be much more difficult for a wild living species.

Most isolates obtained in this study (78.1%) have been identified as *stx2b* subtype. This result corroborates to previously published German (Lehmann et al., 2006; Eggert et al., 2012) and Spanish (Mora et al., 2012) studies. The subtype *stx2c* including *stx2d* was identified in two isolates of one roe deer. *Stx2c* is associated with HUS and HC cases in humans and hence being discussed as a virulence gene with high human pathogenicity (Friedrich et al., 2002). *Stx1c* was detected in 19 isolates (15.3%) of four animals and in one isolate of one animal together with the subtype *stx1a*. In some studies *stx1c* got only rarely detected (Leotta et al., 2006; Ishii et al., 2007; Eggert et al., 2012). Mora et al. (2012) described comparable occurrences: 11.5% of roe deer isolates were *stx1c*-positive, *stx1c* occurred in combination with *stx2b* in 19% and *stx1a* in 3.8%. The *e-hlyA* detection rate of 44.8% found in this study is comparable to previously described rates of 38% in roe deer in this area (Eggert et al., 2012) and in 33% of German roe deer, red deer and fallow deer (Lehmann et al., 2006). All isolates lacked the *eae* gene and only six isolates (5.8%) carried the *saa* gene in this study. STEC isolates of deer investigated by Eggert et al. (2012) were devoid *eae* negative and 9% *saa* positive. However, in a previous study *saa* had been detected at a much higher rate (87%) in deer (Ishii et al., 2007). Likewise, STEC isolates of deer investigated by Leotta et al. (2006) and Sánchez et al. (2009) were devoid of the *eae* and the *saa* gene. In Spain, 6.7% isolates from roe deer (7/104) carried the *eae* gene (Mora et al., 2012). Also Obwegeser et al. (2012) found no *stx*-positive isolate from roe deer with *eae* in Switzerland. According to our results, roe deer in this region of Germany may mainly be regarded as a reservoir for *eae*-negative STEC, often with the subtype *stx2b* and frequently possessing *e-hlyA*.

It was not possible to assign any of the isolates to one of the 5 tested serogroups. These results are similar to those of other studies, characterizing STEC isolated from deer and deer meat (Lehmann et al., 2006; Ishii et al., 2007; Eggert et al., 2012). Further studies showed for less than 1% of the examined isolates from deer and deer meat serogroups of O26, O103, O145 and/or O157 (Miko et al., 2009; Mora et al., 2012; Singh et al., 2015). The five most common serogroups associated with human disease appear to be rare at roe deer in this region.

The isolates were all sensitive against all antimicrobial substances tested. There are only a few studies on STEC resistance rates in wild ruminants. Drees (2012) found one isolate with lower susceptibility to streptomycin among 68 examined deer isolates (1.5%). In Asakura et al. (1998), all investigated STEC isolates (n = 7) from free-ranging game were completely sensitive to the tested antimicrobial substances. In Belgium, 15 STEC isolates from 9 roe deer were analysed using the disc diffusion method. All isolates were at least intermediate sensitive to one up to three out of eight antimicrobial substances. Two isolates of one animal were additionally resistant to spectinomycin (Bardiau et al., 2010). A reason for the low resistance frequencies of the roe deer examined in this study could be due to the fact that the wild ruminants in the Bavarian Forest National Park did not receive any antibiotic treatment throughout their lifetime and generally have little contact with humans or cattle.

The isolates could be assigned to the phylogenetic groups B1 (39.6%) and B2 (60.4%). All *stx1c*-positive isolates belong to the phylogenetic group B1 and had almost identical band patterns in PFGE (Table 2–3). Ishii et al. (2007) found only *stx1c* isolates which could also be assigned to group B1. In Spain 104 isolates of roe deer could be assigned to the phylogenetic groups B1 (47%), B2 (21%) and D (32%). They found B1 and B2 significantly more often in deer as in wild boar (Mora et al., 2012). Group B2 were described in a previous study as typically associated to extraintestinal pathogenic *E. coli* strains (Clermont et al., 2000; Mora et al., 2009; Clermont et al., 2013). Deer living in the examined regions seem to be frequently carrier of STEC of the phylogenetic group B2. All tested animals were clinically unremarkable.

## 5. Conclusion

Although the majority of roe deer were *stx*-positive whenever sampled, for none of the isolated STEC *eae* or serogroups most commonly associated with human disease were detected. In addition, considering that *stx*-subtype most often found was *stx2b*, it appears that strains harboured by this population of roe deer represent a relatively low pathogenic potential to humans. Nevertheless, the animals could be carriers for several strains, simultaneously or consecutively and only a few roe deer were *stx*-negative, whenever sampled. Interestingly, the STEC strains "*stx2b*, phylogenetic group B2, PFGE types B and P" and "*stx1c*, *ehlyA*-positive, phylogenetic group B1, PFGE type M" were repeatedly found over long periods of time. Although the duration of each

shedding event could not be determined the results indicate possibly long-term excretion of STEC in roe deer. This is an important finding, underlining the need of good hygienic practice in the course of field dressing of deer and preparing deer meat.

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## Declaration of Competing Interest

None to declare.

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## References

Asakura, H., Makino, S., Shirahata, T., Tsukamoto, T., Kurazono, H., Ikeda, T., Takeshi, K., 1998. Detection and genetical characterization of shiga toxin-producing *Escherichia coli* from wild deer. Microbiol. Immunol. 42, 815–822. https://doi.org/10.1111/j.1348-0421.1998.tb02356.x.

Bardiau, M., Grégoire, F., Muylaert, A., Nahayo, A., Duprez, J., Mainil, J., Linden, A., 2010. Enteropathogenic (EPEC), enterohaemorragic (EHEC) and verotoxigenic (VTEC) *Escherichia coli* in wild cervids. J. Appl. Microbiol. 109, 2214–2222. https://doi.org/10.1111/j.1365-2672.2010.04855.x.

Beutin, L., Fach, P., 2014. Detection of shiga toxin-producing *escherichia coli* from non-human sources and strain typing. Microbiolspec 2. https://doi.org/10.1128/microbiolspec.EHEC-0001-2013.

Ciuti, S., Tripke, H., Antkowiak, P., Silveyra Gonzalez, R., Dormann, C.F., Heurich, M., 2018. An efficient method to exploit LiDAR data in animal ecology. Methods Ecol. Evol. 9, 893–904. https://doi.org/10.1111/2041-210X.12921.

Clermont, O., Bonacorsi, S., Bingen, E., 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. 66, 4555–4558. https://doi.org/10.1128/AEM.66.10.4555-4558.2000.

Clermont, O., Christenson, J.K., Denamur, E., Gordon, D.M., 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ. Microbiol. Rep. 5, 58–65. https://doi.org/10.1111/1758-2229.12019.

Coura, F.M., de Araújo Diniz, S., Mussi, J.M.S., Silva, M.X., Lage, A.P., Heinemann, M.B., 2017. Characterization of virulence factors and phylogenetic group determination of *Escherichia coli* isolated from diarrheic and non-diarrheic calves from Brazil. Folia Microbiol. (Praha) 62, 139–144. https://doi.org/10.1007/s12223-016-0480-9.

Dabke, G., Le Menach, A., Black, A., Gamblin, J., Palmer, M., Boxall, N., Booth, L., 2014. Duration of shedding of Verocytotoxin-producing *Escherichia coli* in children and risk of transmission in childcare facilities in England. Epidemiol. Infect. 142, 327–334. https://doi.org/10.1017/S095026881300109X.

Drees, N., 2012. Untersuchung auf antimikrobielle Resistenzen und Vorkommen von Extended-Spectrum-Beta-Lactamasen bei Shigatoxin-Gen tragenden *Escherichia coli* (in German, with English abstract). Dissertation. Tierärztliche Fakultät, LMU München. https://nbn-resolving.org/urn:nbn:de:bvb:19-148880.

Eggert, M., Stüber, E., Heurich, M., Fredriksson-Ahomaa, M., Burgos, Y., Beutin, L., Märtlbauer, E., 2012. Detection and characterization of Shiga toxin-producing *Escherichia coli* in faeces and lymphatic tissue of free-ranging deer. Epidemiol. Infect. 141, 251–259. https://doi.org/10.1017/S0950268812000246.

European Food Safety Authority (EFSA), 2009. Scientific Report of EFSA - Technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food. Efsa J. 7, 1366. https://doi.org/10.2903/j.efsa.2009.1366.

European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC), 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. Efsa J. 16, 87–113. https://doi.org/10.2903/j.efsa.2018.5500.

Ferdous, M., Friedrich, A.W., Grundmann, H.P., Boer, R.F., Croughs, P.D., Islam, M.A., Bergh, M.F., Kooistra-Smid, A.M., Rossen, J.W., 2016. Molecular characterization and phylogeny of Shiga toxin-producing *Escherichia coli* isolates obtained from two Dutch regions using whole genome sequencing. Clin. Microbiol. Infect. 22, 642. https://doi.org/10.1016/j.cmi.2016.03.028. e1-e9.

Fischer, J.R., Zhao, T., Doyle, M.P., Goldberg, M.R., Brown, C.A., Sewell, C.T., Kavanaugh, D.M., Bauman, C.D., 2001. Experimental and field studies of *Escherichia coli* O157:H7 in white-tailed deer. Appl. Environ. Microbiol. 67, 1218–1224. https://doi.org/10.1128/AEM.67.3.1218-1224.2001.

Friedrich, A.W., Bielaszewska, M., Zhang, W.-L., Pulz, M., Kuczius, T., Ammon, A., Karch, H., 2002. Escherichia coli Harboring Shiga Toxin 2 Gene Variants: Frequency and Association with Clinical Symptoms. J. Infect. Dis. 185, 74–84. https://doi.org/10.1086/338115.

Henderson, H., 2008. Direct and indirect zoonotic transmission of Shiga toxin–producing Escherichia coli. J. Am. Vet. Med. Assoc. 232, 848–859. https://doi.org/10.2460/javma.232.6.848.

Ishii, S., Meyer, K.P., Sadowsky, M.J., 2007. Relationship between phylogenetic groups, genotypic clusters, and virulence gene profiles of *Escherichia coli* strains from diverse human and animal sources. Appl. Environ. Microbiol. 73, 5703–5710. https://doi.org/10.1128/AEM.00275-07.

Kemper, N., Aschfalk, A., Höller, C., 2006. *Campylobacter* spp., *Enterococcus* spp., *Escherichia coli*, *Salmonella* spp., *Yersinia* spp., and *Cryptosporidium oocysts* in semi-domesticated reindeer (*Rangifer tarandus tarandus*) in Northern Finland and Norway. Acta Vet. Scand. 48, 7. https://doi.org/10.1186/1751-0147-48-7.

Laaksonen, S., Oksanen, A., Julmi, J., Zweifel, C., Fredriksson-Ahomaa, M., Stephan, R., 2017. Presence of foodborne pathogens, extended-spectrum β-lactamase -producing *Enterobacteriaceae*, and methicillin-resistant *Staphylococcus aureus* in slaughtered reindeer in northern Finland and Norway. Acta Vet. Scand. 59, 2. https://doi.org/10.1186/s13028-016-0272-x.

Lehmann, S., Timm, M., Steinrück, H., Gallienet, P., 2006. Nachweis von STEC im Kot von Hochwild und in Wildfleischproben (in German, with English abstract). Fleischwirtsch. 4, 93–96.

Leotta, G.A., Deza, N., Origlia, J., Toma, C., Chinen, I., Miliwebsky, E., Iyoda, S., Sosa-Estani, S., Rivas, M., 2006. Detection and characterization of Shiga toxin-producing *Escherichia coli* in captive non-domestic mammals. Vet. Microbiol. 118, 151–157. https://doi.org/10.1016/j.vetmic.2006.07.006.

Lim, J.Y., Yoon, J., Hovde, C.J., 2010. A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. J. Microbiol. Biotechnol. 20, 5–14. https://doi.org/10.4014/jmb.0908.08007.

Matussek, A., Einemo, I.M., Jogenfors, A., Löfdahl, S., Löfgren, S., 2015. Shiga toxin-producing *Escherichia coli* in diarrheal stool of swedish children: evaluation of polymerase chain reaction screening and duration of shiga toxin shedding. J. Pediatric Infect. Dis. Soc. 5, 147–151 https://dx.doi.org/10.1093%2Fjpids%2Fpiv003.

Messelhäusser, U., Fricker, M., Ehling-Schulz, M., Ziegler, H., Elmer-Englhard, D., Kleih, W., Busch, U., 2007. Real-time-PCR-System zum Nachweis von *Bacillus cereus* (emetischer Typ) in Lebensmitteln (in German, with English abstract). J. Verbrauch. Lebensm. 2, 190–193. https://doi.org/10.1007/s00003-007-0172-0.

Miko, A., Pries, K., Haby, S., Steege, K., Albrecht, N., Krause, G., Beutin, L., 2009. Assessment of Shiga toxin-producing *Escherichia coli* isolates from wildlife meat as potential pathogens for humans. Appl. Environ. Microbiol. 75, 6462–6470. https://doi.org/10.1128/AEM.00904-09.

Mora, A., Blanco, J.E., Blanco, M., Alonso, M.P., Dhabi, G., Echeita, A., González, E.A., Bernárdez, M.I., Blanco, J., 2005. Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Res. Microbial. 156, 793–806. https://doi.org/10.1016/j.resmic.2005.03.006.

Mora, A., López, C., Dabhi, G., Blanco, M., Blanco, J.E., Alonso, M.P., Herrera, A., Mamani, R., Bonacorsi, S., Moulin-Schouleur, M., Blanco, J., 2009. Extraintestinal pathogenic *Escherichia coli* O1:K1:H7/NM from human and avian origin: detection of clonal groups B2 ST95 and D ST59 with different host distribution. BMC Microbiol. 9, 132. https://doi.org/10.1186/1471-2180-9-132.

Mora, A., López, C., Dhabi, G., López-Beceiro, A.M., Fidalgo, L.E., Díaz, E.A., Martínez-Carrasco, C., Mamani, R., Herrera, A., Blanco, J.E., Blanco, M., Blanco, J., 2012. Seropathotypes, Phylogroups, Stx subtypes, and intimin types of wildlife-carried, shiga toxin-producing *escherichia coli* strains with the same characteristics as human-pathogenic isolates. Appl. Environ. Microbiol. 78, 2578–2585. https://doi.org/10.1128/AEM.07520-11.

Möst, L., Hothorn, T., Müller, J., Heurich, M., 2015. Creating a landscape of management: unintended effects on the variation of browsing pressure in a national park. For. Ecol. Manag. 338, 46–56. https://doi.org/10.1016/j.foreco.2014.11.015.

Nielsen, E.M., Andersen, M.T., 2003. Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5′ nuclease PCR assay. J. Clin. Microbiol. 41, 2884–2893. https://www.dx.doi.org/10.1128%2FJCM.41.7.2884-2893.2003.

Obwegeser, T., Stephan, R., Hofer, E., Zweifel, C., 2012. Shedding of foodborne pathogens and microbial carcass contamination of hunted wild ruminants. Vet. Microbiol. 159, 149–154. https://doi.org/10.1016/j.vetmic.2012.03.031.

Sánchez, S., García-Sánchez, A., Martínez, R., Blanco, J., Blanco, J.E., Blanco, M., Dahbi, G., Mora, A., Hermoso de Mendoza, J., Alonso, J.M., Rey, J., 2009. Detection and characterisation of Shiga toxin-producing *Escherichia coli* other than *Escherichia coli* O157:H7 in wild ruminants. Vet. J. 180, 384–388. https://doi.org/10.1016/j.tvjl.2008.01.011.

Scheutz, F., Teel, L.D., Beutin, L., Piérard, D., Buvens, G., Karch, H., Mellmann, A., Caprioli, A., Tozzoli, R., Morabito, S., Strockbine, N.A., Melton-Celsa, A.R., Sanchez, M., Persson, S., O'Brien, A.D., 2012. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. J. Clin. Microbiol. 50, 2951–2963. https://www.dx.doi.org/10.1128%2FJCM.00860-12.

Sharma, V.K., Dean-Nystrom, E.A., 2003. Detection of enterohemorrhagic *Escherichia coli* O157:H7 by using a multiplex real-time PCR assay for genes encoding intimin and Shiga toxins. Vet. Microbiol. 93, 247–260. https://doi.org/10.1016/S0378-1135(03)00039-7.

Singh, P., Sha, Q., Lacher, D.W., Del Valle, J., Mosci, R.E., Moore, J.A., Scribner, K.T., Manning, S.D., 2015. Characterization of enteropathogenic and Shiga toxin-producing *Escherichia coli* in cattle and deer in a shared agroecosystem. Front. Cell. Infect. Microbiol. 5, 29. https://doi.org/10.3389/fcimb.2015.00029.