

Detection and characterization of Shiga toxin-producing *Escherichia coli* in faeces and lymphatic tissue of free-ranging deer

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Received 12 October 2011; Final revision 26 January 2012; Accepted 1 February 2012;
first published online 28 February 2012

SUMMARY

Shiga toxin-producing *Escherichia coli* (STEC) have led to outbreaks worldwide and are considered emerging pathogens. Infections by STEC in humans have been reported after consumption of mainly beef, but also deer. This study investigated the occurrence of STEC in deer in Germany. The virulence genes *eae*, *e-hlyA* and *saa*, the *stx* subtypes, pulsed-field gel electrophoresis (PFGE) patterns and serovars were studied. In total, 120 samples of 60 animals were screened by real-time polymerase chain reaction (PCR). The PCR results showed a high detection rate of *stx* genes (83%). Mainly faecal samples, but also some lymphatic tissue samples, tested *stx*-positive. All isolates carried *stx2*, were *eae*-negative and carried *e-hlyA* in 38% and *saa* in 9% of samples. Serovars (O88:[H8], O174:[H8], O146:H28) associated with human diseases were also identified. In some animals, isolates from lymphatic tissue and faecal samples showed undistinguishable PFGE patterns. The examined deer were shown to be relevant reservoirs of STEC with subtype *stx2b* predominating.

Key words: Bacterial typing, Shiga toxin-producing *E. coli*.

INTRODUCTION

More than 3500 Shiga toxin-producing *Escherichia coli* (STEC) cases in humans were confirmed in European countries in 2009 [1]. The pathogenicity of *E. coli* for humans probably depends on the virulence

profile of the bacteria. The Shiga toxins (Stx) 1 and 2, encoded by their respective genes, rank among the most potent bacterial toxins. The *stx* genes can be classified into subtypes *stx1a*, *stx1c*, *stx1d* and *stx2a–stx2g* [2]. Stx2 compared to Stx1 seems to lead to more severe diseases in humans [3]. Another major virulence factor of STEC is intimin, encoded by the *eae* gene. In patients suffering from haemorrhagic colitis (HC) or the life-threatening haemolytic uraemic syndrome (HUS), it is mostly STEC isolates

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carrying the *eae* gene as well as the *e-hlyA* gene, encoding the pore-building cytolysin enterohaemolysin, that have been detected [4]. The STEC auto-agglutinating adhesin gene (*saa*) was first discovered 2001 in relation to a HUS outbreak in Australia and might represent an additional virulence gene particularly for *eae*-negative STEC [5].

Not only is the virulence profile of great interest, but also the serovar of STEC strains. Currently, a large number of different serovars have been determined to be associated with human diseases [6]. The serovar O157:H7 was found to be the main serovar causing STEC outbreaks in the USA and Europe [7]. However, the importance of non-O157 serovars for human diseases has been underestimated due to unawareness of the involvement of non-O157 in human diseases and the lack of appropriate culturing methods [8].

Transmission of STEC strains is possible via direct contact with humans or animals, but mainly occurs via consumption of STEC-contaminated food products [9]. So far, STEC strains have been detected in many animals, e.g. turkeys, pigs and horses [7, 10]. However, ruminants, in particular cattle are regarded as the main reservoir of this pathogen [7, 8]. More than 400 different STEC serovars have been isolated from healthy cattle, indicating that STEC belong to the intestinal flora of cattle [11, 12]. Beutin *et al.* were able to isolate strains of the same serovar from cattle as well as from patients with diarrhoea or HUS, thus confirming the role of cattle in human infections [13]. However, other ruminants may also represent important reservoirs [6, 14]. Thus far, few studies have dealt with the occurrence and characterization of STEC in cervids in Europe [14–18] and no study has ever investigated STEC in lymphatic tissue of deer. The objective of our study was to investigate the occurrence of STEC in lymphatic tissue and faeces of red and roe deer and to characterize the virulence profile, serovar and the pulsed-field gel electrophoresis (PFGE) pattern of the isolates.

METHODS

Samples

In total, 120 samples from healthy red (*Cervus elaphus*) and roe (*Capreolus capreolus*) deer were taken in the Bavarian Forest National Park. Red deer sampling comprised 30 faecal samples, 30 mesenterial lymph node samples and 30 tonsil samples from 30 animals, shot for livestock control. To minimize the

risk of cross-contamination the lymph nodes and tonsils were aseptically removed with sterile instruments and separately stored in sterile bags, before collecting the corresponding faecal sample. Thirty faecal samples were collected from 30 live roe deer from the ground of a live catch trap. Of the 30 red deer 20 were female and 10 male with an age range from 4 months to 12 years. Data concerning the sex and age of the 30 roe deer could not be obtained from all animals. Ten female and 10 male roe deer with an age range from a few months to 8 years were registered. A description of the research area and the wildlife management can be found in Heurich *et al.* [19].

Bacterial strains

E. coli reference strains were used as positive controls for the investigated virulence genes. The strains C600J1 (*stx1a*), C600W34 (*stx2a*) and LGL 2010/02 (*saa*) were kindly provided by the National Reference Laboratory for *Escherichia coli* at the BfR, Berlin, Germany, and by the Bavarian Health and Food Safety Agency, Oberschleißheim, Germany. The remaining strains WK 184ON (*stx1c*), MHI 813 (*stx1d*), MHI 830 (*stx2b*), MHI 829 (*stx2c*), MHI 831 (*stx2e*), MHI 832 (*stx2f*), MHI 834 (*eae*) and MHI 820 (*e-hlyA*) were obtained from the collection of the Institute of Food Science, Munich, Germany.

Primers for real-time polymerase chain reaction (PCR)

The primers used in this work are listed in Table 1. Primers for *stx1c*, *stx1d*, *stx2b*, *stx2c*, *stx2e* and *stx2f* had been designed for conventional PCR. In this study, protocols for these primers were established for real-time PCR.

Detection of *stx* genes

The mesenterial lymph nodes (10 g), tonsils (10 g) and faecal samples (1 g) were each enriched in 90 ml buffered peptone water (BPW; Merck, Germany) for 20 h at 37 °C. After overnight enrichment DNA extraction was performed from 100 µl of lymph node and tonsil broth with InstaGene™ Matrix (Bio-Rad, Germany) according to the manufacturer's protocol. Direct DNA extraction from the overnight enrichment of faecal samples was not possible due to inhibitory factors. Hence, one loop of the faecal overnight enrichment was streaked onto sorbitol-MacConkey agar plate (SMAC; Merck) and incubated for 24 h at

Table 1. Primers for the detection of STEC virulence genes by real-time PCR

Targets	Primer	Oligonucleotide sequence (5'–3')	PCR product size (bp)	Ref.
<i>stx1</i>	stx1f	GACTGCAAAGACGTATGTAGATTCG	150	[20]
	stx1r	ATCTATCCCTCTGACATCAACTGC		
<i>stx2</i>	stx2f	ATTAACCACACCCACCG	210	[20]
	stx2r	GTCATGGAAACCGTTGTAC		
<i>stx1c</i>	Lin-up	GAACGAAATAATTTATATGT	555	[21]
<i>stx1d</i>	1ox3	CTCATTAGGTACAATTCT	192	[22]
	VT1AvarF	CTTTTCAGTTAATGCGATTGCT		
<i>stx2b</i>	VT1AvarR	AACCCCATGATATCGACTGC	175	[23]
	stx2d-a	GGTAAAATTGAGTTCTCTAAGTAT		
<i>stx2c</i>	stx2d-b	CAGCAAATCCTGAACCTGACG	124	[23]
	stx2c-a	GCGGTTTTATTTGCATTAGT		
<i>stx2e</i>	stx2c-b	AGTACTCTTTCCGGCCACT	303	[23]
	stx2e-a	ATGAAGTGTATATTGTTAAAGTGGA		
<i>stx2f</i>	stx2e-b	AGCCACATATAAATTATTTTCGT	150	[23]
	stx2f-a	TGTCTTCAGCATCTTATGCAG		
<i>eae</i>	stx2f-b	CATGATTAATTACTGAAACAGAAAC	102	[24]
	eae-F2	CATTGATCAGGATTTTTCTGGTGATA		
<i>e-hlyA</i>	eae-R	CTCATGCGGAAATAGCCGTTA	142	[24]
	ehc-F	CGTTAAGGAACAGGAGGTGTCAGTA		
<i>saa</i>	ehc-R	ATCATGTTTTCCGCCAATGAG	85	[24]
	Saa-F	TGCCGCTGGTATAATTTTTTCG		
	Saa-R	AACGCCTGTTCCATGTTGTG		

41.5 °C. The DNA from the faecal samples was extracted from the first streaking area of each SMAC agar plate. Next, the material was suspended in 100 µl molecular biology grade water and 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 *stx1* and *stx2* was conducted in 20 µl volumes containing 10 µl SsoFast™ EvaGreen® Supermix (Bio-Rad), 200 nm of each primer pair (stx1f and stx1r; stx2f and stx2r), 4 µl molecular biology grade water and 2 µl template from overnight enrichment (lymph nodes and tonsils), and 5 µl molecular biology grade water and 1 µl template from bacterial culture (faeces). DNA from the strains C600J1 and C600W34 were used as positive controls and molecular biology grade water was used as no template control. The PCR was performed in an iQ5 Cyclor (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.

Isolation of STEC

Corresponding lymph node and tonsil enrichment cultures of *stx*-positive samples were diluted 1:10 000

and faecal cultures 1:100 000. Next, 100 µl of the dilution was plated onto SMAC agar and incubated for 24 h at 41.5 °C. From SMAC agar plates, 4–20 colonies (sorbitol-positive and sorbitol-negative) were randomly chosen and analysed for *stx1* and *stx2* by multiplex real-time PCR. Colonies which were *stx*-positive were further subcultivated. Pure cultures were investigated for *stx1c*, *stx1d*, *stx2b*, *stx2c*, *stx2e*, *stx2f*, *eae*, *e-hlyA* and *saa* by real-time PCR. The assays were conducted in 20 µl volumes containing 10 µl SsoFast™ EvaGreen® Supermix, 200 nm of the respective primer pair (Table 1), 7 µl molecular biology grade water and 1 µl template from pure *stx*-positive cultures. Thermal cycling consisted of denaturation (98 °C for 5 s), annealing and extension [56 °C (*stx1c*: 54 °C) for 15 s], performed in 40 cycle steps. Melting curve analysis ranged from 65 °C to 95 °C with 0.5 °C intervals. *Stx*-positive isolates were confirmed as *E. coli* by API® 20E (bioMérieux, Germany).

Serotyping

The isolates were serotyped at the National Reference Laboratory for *Escherichia coli* at the BfR, Berlin, Germany, as described previously [25]. The flagellin gene (*fliC*) was used for typing non-motile isolates. These H antigens are typed in square brackets.

PFGE

DNA was isolated using CHEF Genomic DNA plug kit (Bio-Rad). The plugs were lysed for 4–6 h at 37 °C in lysozyme solution and overnight at 50 °C in proteinase K solution. The plugs were washed six times in wash buffer before restriction digestion. The DNA was digested overnight with 20 U *XbaI* (New England Biolabs, Germany) enzyme according to the manufacturer's instructions. The restriction fragments were separated through a 1.0% gel (pulsed field certified agarose; BioRad) in 0.5 × Tris-borate EDTA buffer with a CHEF Mapper XA system (Bio-Rad). Lambda Ladder PFG marker (New England Biolabs) was used as a size standard. Pulse times were ramped from 1 to 22 s over 22 h. The gels were stained with ethidium bromide, destained with the running buffer, and photographed with a Gel Doc EQ system (Bio-Rad). The obtained data were analysed by BioNumerics version 6.5 (Applied Maths, Belgium). Percentages of similarity between fingerprints were determined using the band-based Dice coefficient and a 2% band position tolerance. The unweighted pair-group method with arithmetic mean (UPGMA) on a matrix resulting from comparison of PFGE *XbaI* patterns was used for generating the dendrogram.

Statistical analysis

The data were analysed using the program R version 2.11.1 (open source software by R. Ihaka and R. Gentleman) using Fisher's exact test. The test was performed with a bidirectional null hypothesis. A *P* value of <0.05 was considered statistically significant.

RESULTS

Occurrence of *stx* genes

In total, 28 (93%) of 30 red deer were positive for *stx* (Table 2). In 28 *stx*-positive red deer, seven had only *stx*-positive faecal samples. In eight animals *stx* gene was detected in both faecal and lymph node samples, and in two animals in both faecal and tonsil samples. In four animals *stx* was detected in all samples. In four cases, only the lymph node samples, and in two cases lymph node and tonsil samples were positive. In one animal *stx* was only detected in the tonsils. Comparing the three types of samples, in both male and female red deer, *stx* was detected most frequently in faecal samples (8/10 male red deer; 13/20 female

Table 2. Detection of *stx* in red and roe deer by real-time PCR

Species	No. of animals	Sample	No. of samples	No. of <i>stx</i> -positives	Detection rate
Red deer	30			28	93%
		Tonsil	30	9	30%
		Lymph node	30	18	60%
Roe deer	30	Faecal	30	21	70%
		Faecal	30	22	73%
Total	60			50	83%

red deer). Similarly, red deer aged <3 years showed the highest frequency of *stx* in faeces (13/18; 72%), followed by lymph nodes (9/18; 50%) and tonsils (5/18; 28%). Animals aged >3 years tested positive for *stx* in 75% of lymph node samples (9/12), in 67% of faecal samples (8/12) and in 33% of tonsil samples (4/12). In roe deer, *stx* was detected in 22 (73%) out of 30 faecal samples. Seven of 10 male and 9/10 female roe deer carried the *stx* genes. The sex and age of the remaining six *stx*-positive roe deer could not be ascertained. The detection rate of *stx* was not significantly correlated to sex, age or animal species (*P*>0.05).

Isolation of STEC

In total, 32 *stx*-positive isolates were recovered from 25 *stx*-positive red and roe deer. From 15 *stx*-positive red deer, 21 isolates were obtained from faeces, lymph nodes or tonsils (13, 5, 3, respectively). From 10 *stx*-positive roe deer, 11 isolates were obtained from faeces. All isolates were sorbitol-positive.

Virulence gene profile

All 32 *stx*-positive isolates were positive for the *stx2* gene. Two of the 32 isolates also tested positive for *stx1* gene. A clearly higher frequency was shown by *stx2* variant genes compared to *stx1* variant genes. The two *stx1*-positive isolates obtained from red deer could be further subtyped to *stx1c*. Subtyping of the *stx2*-positive isolates resulted in the detection of two different subtypes: *stx2b* and *stx2c*. Of these, *stx2c* was only found in one isolate obtained from roe deer faeces, while *stx2b* was detectable in the remaining 31 (97%) *stx*-positive isolates. None of the isolates tested positive for the subtypes *stx1d*, *stx2e* and *stx2f*.

Table 3. Gene combinations and serovars of the 32 *stx*-positive isolates obtained from red and roe deer

Gene serovar	Total	Isolates obtained from red deer	Isolates obtained from roe deer
<i>stx2b</i>	19	16	3
Orough:H28	6	3	3
O130:[H30]	5	5	0
O130:H30	1	1	0
O146:H28	4	4	0
O21:H21	3	3	0
<i>stx2b + hlyA</i>	8	3	5
O110:[H31]	2	0	2
O146:H28	1	0	1
O18:[H49]	1	1	0
O18:H49	1	1	0
O88:[H8]	1	1	0
O21:H21	1	0	1
Orough:H21	1	0	1
<i>stx1c + stx2b + hlyA</i>	2	2	0
O174:H8	1	1	0
O174:[H8]	1	1	0
<i>stx2b + saa</i>	1	0	1
Orough:H28	1	0	1
<i>stx2b + hlyA + saa</i>	1	0	1
Orough:H21	1	0	1
<i>stx2c + hlyA + saa</i>	1	0	1
O142:H16	1	0	1

[...] = Non-motile strain, *fliC* type analysed by PCR.

The virulence gene *e-hlyA* was present in 12 (38%) isolates. *saa* was detected in three (9%) isolates. None of the isolates carried the virulence gene *eae*. With regard to the gene combination, *stx2b* without any other tested gene was detected most frequently (59%), followed by the combination *stx2b + e-hlyA* (25%). In total, the 21 isolates obtained from red deer comprised three different gene combinations and the 11 isolates obtained from roe deer shared five gene combinations (Table 3).

Analysis of serovars

The 32 *stx*-positive isolates could be assigned to ten different serovars. Here, eight different O types (O18, O21, O88, O110, O130, O142, O146, O174) and seven different H types (H8, H16, H21, H28, H30, H31, H49) were detected. Orough was found in nine isolates. The combination Orough:H28 was detected most frequently, in seven (22%) of 32 isolates. Six (19%) isolates harboured the serovar O130:H30, with five

isolates being non-motile. O146:H28 and O21:H21 were detectable in five and four isolates, respectively. O174:H8 was found twice with one isolate being non-motile. Two isolates were serotyped as O110:H31, both non-motile. O18:H49 and Orough:H21 were each detected in two isolates, with one of the O18:H49 isolate being non-motile. An isolate with serovar O142:H16 and a non-motile isolate with the serovar O88:H8 were each found once (Table 3).

PFGE patterns

The 32 isolates yielded 26 different *Xba*I profiles. The 21 isolates recovered from 15 red deer resulted in 16 different profiles. The 11 isolates from 10 roe deer exhibited 10 different patterns. Two red deer possessed isolates with undistinguishable patterns in faeces and lymph nodes or in faeces and tonsils, respectively. Similarly, another red deer harboured isolates with undistinguishable patterns in faecal and lymph node isolates, but the tonsil isolate differed. Twice, isolates with undistinguishable patterns were present in faeces of different animals. In another case, faecal and tonsil isolates of different animals were undistinguishable with *Xba*I (Fig. 1). The isolates could be divided into 11 different groups, based on a similarity of >70% and corresponded to the O types.

DISCUSSION

In contrast to previous studies focusing on the occurrence of the serovar O157:H7 [26, 27], this study deals with the occurrence of STEC in deer irrespective of the serovar. Over 70% of the deer excreted *stx*-positive faeces and of 42% of the deer STEC could be isolated. In a previous German investigation, STEC isolates were recovered from 52% of roe, red, and fallow deer faecal samples. The samples were taken from five different hunting grounds [17]. In Argentina, faeces of captive wild deer were found to be *stx*-positive in 38% of samples [28]. Studies of STEC in faeces from non-captive deer performed in other countries mainly revealed clearly lower occurrences. In Spain, STEC were identified in 25% of red deer and in 5% of roe deer [14] by testing randomly chosen colonies from the samples. In Belgium, STEC were only present in 12% of roe and red deer [15]. Furthermore, only 10% of free-ranging deer and 16% of wild deer in Japan excreted STEC in their faeces [29, 30]. Lillehaug *et al.* were unable to detect STEC in Norwegian roe deer, and in red deer only

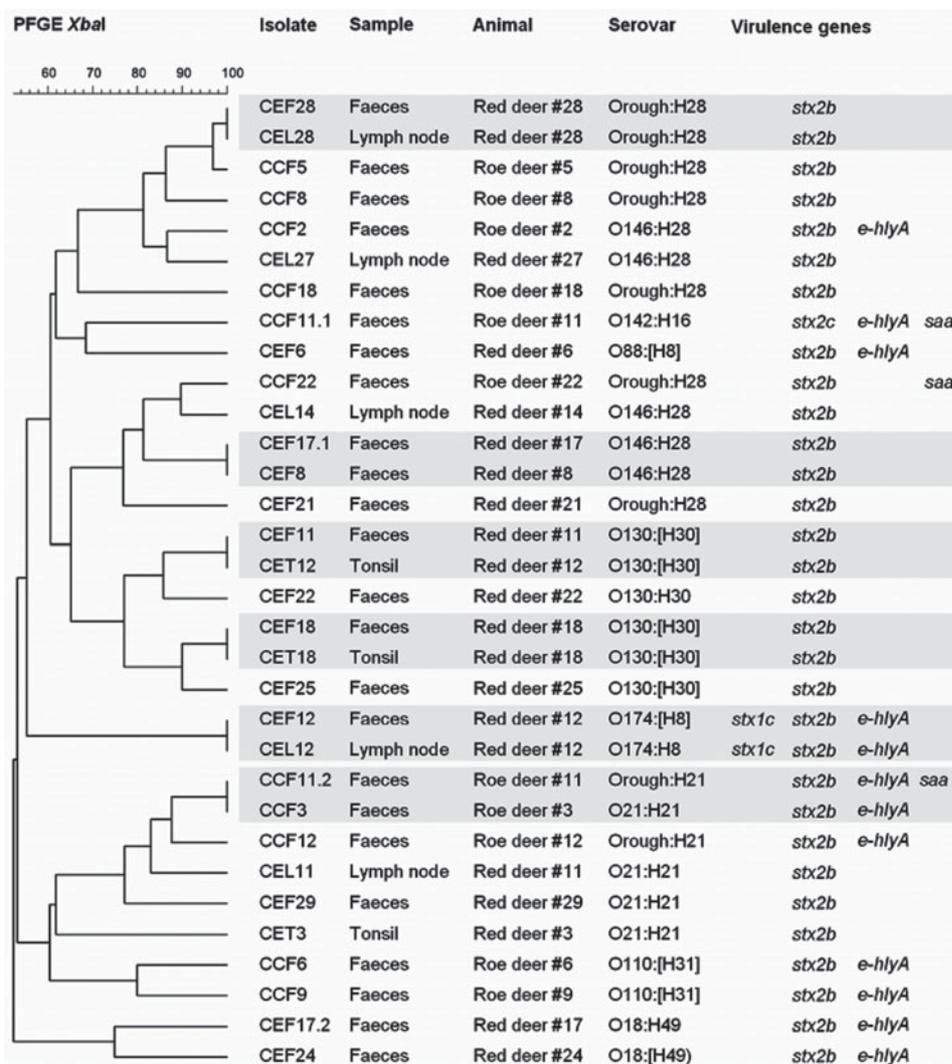


Fig. 1. Dendrogram analysis of STEC strains isolated from red and roe deer in Germany. The isolate names, serovars, and virulence genes are indicated. The tree was constructed by BioNumerics software v. 6.5 using the Dice coefficient (tolerance 2%) and UPGMA on a matrix resulting from comparison of PFGE *XbaI* patterns. CC, *Capreolus capreolus* (roe deer); CE, *Cervus elaphus* (red deer). The scales at the top indicate the similarity indices (in percentages). Isolates with undistinguishable PFGE *XbaI* patterns are highlighted in grey.

2/135 individuals tested *stx*-positive, after screening with immunomagnetic separation for the O types: O26, O103, O145, O111 and O157 [18].

The 60 investigated deer originated from the Bavarian Forest National Park. As these deer are sometimes fed during winter, the high level of *stx*-positive animals could be due to a crowding effect at the feeding sites. In this way, a transmission of STEC is likely via oral ingestion of food contaminated with *stx*-positive faeces. This suggestion is supported by the isolation of identical isolates in tonsils and faeces of both the same and different animals. Another explanation for the high occurrence of STEC in deer could be that the sampled animals belonged mainly

to the same flock. However no data are available to assign each sampled deer to its flock. In spite of sampling the deer randomly and at different places in the national park, a selection bias cannot be ruled out due to the small sample size. All sampled animals showed no clinical symptoms, implying that deer function as asymptomatic STEC carriers and excretors, like cattle. No cases of disease or death in deer caused by STEC could be found in the literature.

Three different sample types (faeces, lymph nodes, tonsils) were obtained from red deer. To our knowledge, this is the first study investigating the occurrence of STEC in lymph nodes and tonsils of deer. In an

Italian study, lymph nodes and tonsils of cattle were tested for the serovars O26, O103, O111, O145 and O157. One of 89 lymph node and 1/93 tonsil samples were positive for O157 [31]. In this study, 18 (60%) of 30 and nine (30%) of 30 red deer were *stx*-positive in lymph nodes or tonsils, respectively. As a consequence, our study demonstrates that STEC can also be found in lymphatic tissue of deer, indicating that STEC in deer is not restricted to the intestinal tract. Previous studies in animal models proved that bacteria, e.g. *E. coli* and *Salmonella Gallinarum*, are able to translocate from the intestine to mesenteric lymph nodes [23, 32]. Thus, an explanation for STEC being present in mesenteric lymph nodes could be that STEC might possess the ability of migrating across the intestinal barrier. This suggestion is supported by the finding that in most cases, the STEC isolated from faeces and lymph nodes of the same animal were of the same serovar and exhibited an identical PFGE profile.

Isolates with undistinguishable PFGE patterns indicate that different samples and animals can harbour the same strains. However, these strains were each only found within the same species, whereas Leotta *et al.* described an inter-species transmission of STEC strains between different species of captive non-domestic ruminants [28]. This could be due to differences in habitats or due to the limited number of isolates in this study.

All 32 isolates carried *stx2*, mostly *stx2b*. Asakura *et al.* also detected only *stx2*-positive deer in Japan, but did not differentiate between *stx2* subtypes [29]. However, another Japanese study revealed a higher rate of *stx1* (48%) than of *stx2* (24%) in deer without differentiating between the *stx* gene subtypes [30]. With respect to the *stx2* subtypes, *stx2b* predominated with 97% in our samples. This result corroborates a previously published German study [17]. The subtype *stx2c* was identified in one isolate of roe deer. *stx2c* is associated with HUS and HC cases in humans, hence being discussed as a virulence gene of high pathogenicity for humans [3, 33]. *stx1c*, harboured by two red deer isolates, had seldom been detected in deer before [28, 34]. Thus, STEC carrying *stx1c* seem to occur rarely in deer. Comparable to the *e-hlyA* detection rate of 38% found in this study, the *e-hlyA* gene had previously been described in 33% of German deer [17]. These findings correspond to our results and indicate that STEC possessing *e-hlyA* can frequently be found in deer. Only three (9%) isolates carried the *saa* gene in this study. However, in

a previous study *saa* had been detected at a much higher rate in deer [34]. All isolates lacked the *eae* gene. Similarly, STEC isolates of deer investigated by Leotta *et al.* [28] and Sánchez *et al.* [14] were devoid of *eae* and *saa* genes. According to our results, red and roe deer in this region of Germany may mainly serve as a reservoir for *saa*-negative and *eae*-negative STEC.

The serovar combinations O146:H28, O21:H21, O174:H8, O110:H31 and O88:H8 found in this study, had sporadically been recovered from deer and deer meat before [17, 34, 35]. With regard to the pathogenicity for humans, the serovars O88:H8 and O146:H28 have been isolated from HUS and HC patients in Germany [4]. The serogroups O157 and O26 which mainly account for STEC infections in Germany were not detected in this study [36]. However, serogroups O142, O146 and O174 have been recorded with STEC infections in Germany in the last 2 years [36]. Therefore, considering both the serovar and the virulence gene profile of the 32 isolates, it cannot be ruled out that some isolates carry a risk for humans and could be emerging pathogens. The isolate O142:H16, possessing the *stx2c*, *e-hlyA* and *saa* genes, might particularly be considered as potentially pathogenic.

It is known that food is a crucial transmission vehicle leading to STEC infection in humans. As our work revealed a high occurrence of STEC in deer, great care is required during evisceration and deer meat processing. Tonsil and especially faecal contamination of deer meat carries the risk of transmitting STEC. Some STEC outbreaks and sporadic cases of HC in humans have already been traced back to STEC-contaminated deer meat as the source of infection [37–39].

In conclusion, our results support the findings of previous studies that, in addition to cattle, deer can be regarded as a relevant reservoir for STEC. Furthermore, this work demonstrates that STEC cannot only be detected and isolated from faeces, but also from lymphatic tissue of deer. Regarding the gene profile of isolated STEC, our findings reveal that *stx2b* alone or in combination with *e-hlyA* possibly predominate in deer in Germany. Some isolates possess serovars and gene combinations which have been associated with STEC infections in humans. With the detection of the subtype *stx2c* in deer, our study provides further evidence that deer can be considered as a potential source of STEC infection in humans.

ACKNOWLEDGEMENTS

We thank Dr Ute Messelhäuber for providing the LGL 2010/02 strain. We thank the team from the Bavarian Forest National Park, Germany, especially M. Penn, H. Burghart and L. Ertl.

DECLARATION OF INTEREST

None.

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