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ORIGINAL ARTICLE

Enteropathogenic (EPEC), enterohaemorragic (EHEC) and verotoxigenic (VTEC) *Escherichia coli* in wild cervids

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Abstract

Aims: The aim of this study was to investigate the presence of enteropathogenic (EPEC), enterohaemorragic (EHEC) and verotoxigenic (VTEC) *Escherichia coli* strains in free-ranging wild ruminants in Belgium and to characterize the positive isolates (serogroups and virulence-associated factor-encoding genes).

Methods and Results: *Escherichia coli* strains isolated from faeces of wild cervids were characterized by PCR targeting genes coding for the main virulence properties of EPEC, EHEC and VTEC strains. The prevalence rate of these pathogenic strains in faecal samples obtained from the wild ruminants was found to be 15%. No pathogenic isolate was found to belong to the O157, O26, O111, O103 or O145 serogroups. Moreover, a new gene, *eibH*, showing 88% identity with *eibG* was detected in VTEC strains.

Conclusions: The results reveal that wild ruminants could be considered as a potential source of VTEC and EPEC infection for humans and possibly also for domestic ruminants.

Significance and Impact of the Study: Our study suggests the potential risk of transmission of VTEC, EHEC and EPEC strains from wild ruminants to humans via the consumption of venison and to domestic ruminants because of sharing of the same pasture. Indeed, many serogroups other than O157 EHEC have also been shown to be responsible for outbreaks in humans in several countries, and studies focusing solely on O157:H7 EHEC tend to underestimate this risk of transmission.

Introduction

Enteropathogenic (EPEC), enterohaemorragic (EHEC) and verotoxigenic (VTEC) *Escherichia coli* represent three important classes of enteric pathogens that can cause enteritis and enterotoxaemia in humans and animals. They are characterized by two main virulence properties: (i) the production of histological and ultra-structural lesions called 'attaching and effacing lesions' (A/E lesions) and (ii) the production of Shiga toxins (Stx) (also called verotoxins) (Nataro and Kaper 1998). A/E lesions are characterized firstly, by the effacement of the enterocyte microvilli, as a consequence of cytoskeleton rearrangements initiated by type III-secreted (T3S) bacterial effectors and secondly, by the intimate (<10 nm) attachment of the bacteria to the host enterocytes, via the interaction between an outer membrane protein called intimin (encoded by the *eae* gene) and one of the T3S effectors called Tir (after translocated intimin receptor) (Chen and Frankel 2005; Spears *et al.* 2006). The Shiga toxins are lethal for eukaryotic cells both *in vitro* (Vero, HeLa and/or MDBK cells) and *in vivo* (endothelial cells), because of their effect of blocking protein synthesis (Konowalchuk *et al.* 1977). The Stx toxins belong to one of two families: Stx1 or Stx2. There are several variants of these toxins, particularly within the Stx2 family (Mainil and Daube 2005).

The main virulence property of EPEC strains is the production of A/E lesions, causing the occurrence of an inflammatory reaction and of diarrhoea (Moon *et al.* 1983).

EPEC strains are a major cause of infantile diarrhoea in developing and developed countries and are responsible for thousands of deaths worldwide (Chen and Frankel 2005; Ochoa *et al.* 2008). They are also associated with diarrhoea in most domestic animals species. In cattle, they are associated with diarrhoea in young calves from 1 to 8 weeks of age (China *et al.* 1998).

The main virulence property of VTEC strains is the production of Stx. VTEC strains cause clinical syndromes mainly in humans and piglets. But they can also be isolated from a wide range of domestic and wild animals, which may be asymptomatic healthy carriers (Wieler and Bauerfeind 2003). VTEC infections are less common in humans but, when they do occur, they are frequently associated with haemolytic uraemic syndrome (HUS) (Mainil and Daube 2005). VTEC strains are also responsible for oedema disease in piglets up to 2 weeks after weaning (Moxley 2000).

EHEC strains share the same main virulence properties as the EPEC and VTEC strains, that is the production of A/E lesions and Stx. Nowadays, EHEC strains are considered to have evolved from EPEC strains through the acquisition of bacteriophages encoding Stx (Reid *et al.* 2000) (Wick *et al.* 2005). EHEC strains can cause various syndromes in humans: undifferentiated diarrhoea, haemorrhagic colitis and HUS (Raffaelli *et al.* 2007) and have been associated with significant disease outbreaks in developed countries (USA, Canada, United Kingdom, France, Japan, etc.) in recent years (Stirling *et al.* 2007; Sonoda *et al.* 2008). In several cases, disease has been shown to occur via the consumption of vegetal and animal foodstuffs contaminated by ruminant faeces (mainly cattle) (Erickson and Doyle 2007). Some EHEC strains have also been shown to be responsible for undifferentiated diarrhoea in young calves up to 3 months of age (Mainil and Daube 2005).

Domestic ruminants (especially cattle) are considered to be the main reservoir of EHEC strains for human infection (Hancock *et al.* 2001; Mainil and Daube 2005). Nevertheless, wild ruminants are also considered to represent a potential source of infection for humans and possibly also for domestic ruminants (Pierard *et al.* 1997; Rabatsky-Ehr *et al.* 2002; Simpson 2002). Most studies focus on O157 EHEC strains (Fischer *et al.* 2001; Renter *et al.* 2001; Kemper *et al.* 2006; Heuvelink *et al.* 2008), despite the fact that EHEC strains can belong to a number of different serotypes, many of which are as dangerous to humans as the O157:H7 EHEC, such as the O26, O103, O111 and O145 strains (Campos *et al.* 2004). Only a few studies on wild ruminants do not focus their research on O157 EHEC strains (Asakura *et al.* 1998; Fukuyama *et al.* 1999; Sanchez *et al.* 2009).

The aim of this study was to investigate the presence of EPEC, EHEC and VTEC strains in free-ranging wild ruminants in Belgium using PCR targeting genes coding for the main virulence properties, that is A/E lesions and Stx. The positive isolates were further characterized by PCR for other virulence-associated factor-encoding genes and for five of the most important EHEC somatic antigens (O26, O103, O111, O145 and O157). The positive isolates were also tested for their antibiotic resistance profiles against eight frequently used antibiotics in bovine veterinary medicine.

Materials and methods

Collection of faecal samples and isolation of *Escherichia coli* strains

Through a targeted surveillance programme, the rectal contents of 133 free-ranging wild cervids (*Cervus elaphus* and *Capreolus capreolus*) were collected during the 2008 and 2009 hunting seasons (from 1 October to 31 December) in Wallonia (southern part of Belgium) (Table 1). Individual postmortem examination involved the determination of sex, age (through tooth eruption patterns), body weight and body condition.

The faeces were inoculated onto Gassner agar plates (Merck, Whitehouse Station, NJ, USA) and incubated for 18 h at 37°C. Subsequently, three lactose-fermenting colonies per sample were randomly picked up and transferred into Luria–Bertani (LB) broth (Invitrogen, Carlsbad, CA, USA) with 0·1% tryptophan (Sigma-Aldrich, St Louis, MO, USA). Bacteria were grown for 8 h at 37°C, and Kovacs reagent (Merck) was then added to detect indol production (underlining the action of tryptophanase). Only the isolates that were positive in the indol test were stored at −80°C in 20% glycerol until further characterization.

Genotypic characterization

DNA extraction was carried out by a boiling method as described previously by China *et al.* (1996). Briefly, a pure bacterial culture was grown for 8 h at 37°C in LB broth with slight agitation. Three hundred microlitres of culture was centrifuged for 1 min at 13 000 rev min^{-1}, and the supernatant was discarded. After adding 50 μl of sterile water, the suspension was boiled for 10 min. Afterwards, the suspension was centrifuged for 1 min at 13 000 rev min^{-1}, and the supernatant was stored at −20°C.

Genotypic characterization by PCR (Table 2) was performed in three steps: (i) detection of the *eae* gene coding for the intimin adhesin and of the *stx1* and *stx2* genes coding for Stx toxins (China *et al.* 1996); (ii) detection of

Table 1 Distribution of hunter-killed wild cervids sampled [juvenile, <1 year of age; subadult, 1–2 years of age; adults, >1 year of age (*Capreolus capreolus*) or >2 years of age (*Cervus elaphus*)]

	Number of animals	Age group	Number of animals	Gender	Number of animals
Cap. capreolus	52	Juvenile	15	Female	9
				Male	6
		Adult	37	Female	24
				Male	13
C. elaphus	81	Juvenile	26	Female	14
				Male	12
		Subadult	15	Female	10
				Male	5
		Adult	40	Female	18
				Male	22

Table 2 Primers used in this study

Primer name	Sequence (5′–3′)	Target gene	Annealing temp. (°C)	Amplicon size (bp)	References
B52	AGGCTTCGTCACAGTTG	*eae*	50	570	China *et al.* (1996)
B53	CCATCGTCACCAGAGGA				
B54	AGAGCGATGTTACGGTTTG	*stx1*	50	388	China *et al.* (1996)
B55	TTGCCCCCAGAGTGGATG				
B56	TGGGTTTTTCTTCGGTATC	*stx2*	50	807	China *et al.* (1996)
B57	GACATTCTGGTTGACTCTCTT				
wzx-wzyO26-F	AAATTAGAAGCGCGTTCATC	*wzxO26*	56	596	Durso *et al.* (2005)
wzx-wzyO26-R	CCCAGCAAGCCAATTATGACT				
wzxO157-F	CGGACATCCATGTGATATGG	*rfbO157*	60	259	Paton and Paton (1998)
wzxO157-R	TTGCCTATGTACAGCTAATCC				
wzxO111-F	TAG AGA AAT TAT CAA GTT AGT TCC	*wzxO111*	62	406	Paton and Paton (1998)
wzxO111-R	ATA GTT ATG AAC ATC TTG TTT AGC				
wzxO103-F	TTGGAGCGTTAACTGGACCT	*wzxO103*	57	321	Fratamico *et al.* (2005)
wzxO103-R	GCTCCCGAGCACGTATAAG				
wzxO145-F	CCATCAACAGATTTAGGAGTG	*wzxO145*	59	609	Feng *et al.* (2005)
wzxO145-R	TTTCTACCGCGAATCTATC				
bfpA-F	AATGGTGCTTGCGCTTGCTGC	*bfpA*	56	326	Gunzburg *et al.* (1995)
bfpA-R	GCCGCTTTATCCAACCTGGTA				
EHEC-hlyA-F	ACGATGTGGTTTATTCTGGA	*EHEC-hlyA*	58	165	Fagan *et al.* (1999)
EHEC-hlya-R	CTTCACGTGACCATACATAT				
saa-F	CGTGATGAACAGGCTATTGC	*saa*	50	119	Jenkins *et al.* (2003)
saa-R	ATGGACATGCCTGTGGCAAC				
eibGa-F	ATTTCTTTATGAGTGTGAGGTGTTG	*eibG*	51	552	This study
eibGa-R	CTGTCAGCAATTAAAACTCGAAGTT				
eibGb-F	ATCGGCTTTCATCGCATCAGGAC	*eibG*	60	548	Lu *et al.* (2006)
eibGb-R	CCACAAGGCGGGTATTCGTATC				
eibGc-F	TGTAAAAGACAGTGTTGAGCAACT	*eibG*	51	569	This study
eibGc-R	CGATGAAAGCCGATTGTTTTAA				
SubHCDF	TATGGCTTCCCTCATTGCC	*subA*	60	556	Paton and Paton (2005)
SubSCDR	TATAGCTGTTGCTTCTGACG				

the *bfpA*, *saa* and *eibG* genes coding for other adhesins and of *subA* and *EHEC-hlyA* coding for other toxins produced by different EPEC, EHEC and/or VTEC strains, in the positive isolates; and (iii) genotypic serotyping of the positive isolates. In addition, two new PCRs were designed during this study for the amplification of the 5′ end and of the 3′ end of the *eibG* gene (Table 2). All PCR products were separated by electrophoresis in 1·5% agarose gels. Gels were stained with SYBR Green (Roche Diagnostics Corporation, Basel, Switzerland) and

visualized under UV light. A Fisher's exact test was performed to assess the statistical differences ($P < 0{\cdot}01$) between the different groups of wild cervids (Table 1).

DNA sequencing

DNA fragments were purified using the NucleoSpin Extract II kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. Sequencing of the two DNA strands was performed by the dideoxynucleotide triphosphate chain termination method with a 3730 ABI capillary sequencer and a BigDye Terminator kit version 3.1 (Applied Biosystems, Carlsbad, CA, USA) at GIGA (Groupe Interdisciplinaire de Génoprotéomique Appliquée, University of Liège, Belgium). Sequence analysis was performed using Vector NTI 10.1.1 (Invitrogen, Carlsbad, CA, USA).

Antibiotic susceptibility tests

Susceptibility tests were carried out on the positive isolates for the *eae*, *stx1* and/or *stx2* genes. The tests were performed by the disc diffusion method of Bauer *et al.* (1966) on Mueller–Hinton agar (Oxoid, Hampshire, UK) (Bauer *et al.* 1966). Zones of inhibition were measured (in millimetres) after overnight incubation at 37°C and were interpreted according to the CA-SFM (Comité de l'Antibiogramme de la Société Française de Microbiologie) (Anonymous 2003). Eight antibiotics used widely in bovine veterinary medicine were tested: ceftiofur (30 μg); enrofloxacin (5 μg); the association of trimethoprim–sulfamethoxazole (1·25–23·75 μg); oxytetracycline (30 μg); spectinomycin (100 μg); gentamicin (10 μg); the association of amoxicillin/clavulanic acid (30 μg) (Becton Dickinson, Franklin Lakes, NJ); and florfenicol (30 μg) (Oxoid).

Results

Presence of VTEC, EHEC and EPEC strains

Thirty-seven isolates (37/399, 9·3%) tested positive with at least one of the three initial PCRs: six isolates (1·5%) tested positive with the PCR for the *eae* gene only (EPEC); nine isolates (2·3%) with the PCR for the *stx1* gene only (VTEC); 20 isolates (5%) with the PCR for the *stx2* genes only (VTEC); and two isolates (0·5%) with the PCRs for the *stx1* and *stx2* genes only (VTEC). No isolate tested positive with the PCRs for both the *eae* and the *stx* genes (EHEC). The number of positive isolates varied from one to all three colonies per animal tested (Table 3).

The PCR-positive *E. coli* were isolated from 20 cervids (20/133, 15%). The percentage of carriers did not statistically differ between *C. elaphus* (11/81, 13·6%) and *Cap. capreolus* (9/52, 17·3%), between male (9/58, 15·5%) and female (11/75, 14·7%) animals or between

Table 3 Results obtained for each animal [juvenile, <1 year of age; subadult, 1–2 years of age; adults, >1 year of age (*Capreolus capreolus*) or >2 years of age (*Cervus elaphus*)]

Species	Animal no.	No. of positive strains	Sex	Age	*stx*	*eae*	*eibH*
Cap. capreolus	A09/305	2+/3	F	Adult	1	–	–
	A09/311	1+/3	F	Adult	–	+	–
	A09/325	2+/3	M	Adult	1 and 2	–	–
	A09/332	1+/3	M	Adult	–	+	–
	A09/335	1+/3	F	Juvenile	2	–	–
	A09/337	2+/3	M	Adult	2	–	+
	A09/378	2+/3	M	Adult	2	–	+
	CH09/03	1+/3	M	Juvenile	2	–	–
	CH09/90	3+/3	F	Adult	1	–	–
C. elaphus	A09/299	3+/3	F	Adult	2	–	–
	A09/313	2+/3	F	Adult	2	–	+
	A09/315	2+/3	F	Juvenile	2	–	–
	A09/340	3+/3	F	Adult	2	–	–
	A09/345	2+/3	F	Juvenile	–	+	–
	A09/347	1+/3	M	Juvenile	2	–	–
	A09/371	3+/3	M	Adult	1	–	–
	A09/386	1+/3	F	Subadult	–	+	–
	A10/42	1+/3	M	Juvenile	1	–	–
	C09/73	1+/3	M	Adult	2	–	–
	C09/149	1+/3	F	Adult	–	+	–
		2+/3			2	–	+

adult (13/77, 16·9%) and juvenile/subadult (7/56, 12·5%) animals (Fisher's exact test, $P < 0{\cdot}01$).

Of those 20 cervids, five were found to be carrying EPEC, four were carrying *stx1* + VTEC, 11 were carrying *stx2* + VTEC, and one was carrying *stx1* + /*stx2* + VTEC. One cervid (C09/149) was carrying two different types of strains: two *stx2* + VTEC and one EPEC strain.

Typing of the 37 PCR-positive isolates

All 37 PCR-positive isolates tested negative with the PCRs for the *bfpA*, *saa* and *subA* genes, and only one *stx2* + VTEC from a roe deer tested positive with the PCR for the *EHEC-hlyA* gene (Table 3).

On the other hand, eight *stx2* + VTEC (40% of the *stx2* + VTEC and 26% of all VTEC) isolated from four adult cervids (36·4% of cervids carrying stx2 + VTEC and 25% of cervids carrying VTEC) tested positive with the PCR for the middle of the *eibG* gene using primers eibGb-F and eibGb-R. These amplicons were sequenced for further identification and comparison. The eight amplicon sequences had 100% identity with each other, but only 74% identity with the *eibG* gene (AB255744). Therefore, the 5′ end and the 3′ end of the *eibG* gene were amplified using two other pairs of primers (eibGa-F/eibGa-R and eibGc-F/eibGc-R: Table 2) and were sequenced to obtain the sequence of the entire *eibG*-like gene (1527 bp). The sequence obtained showed 88% identity with the *eibG* gene (AB255744). The accession numbers of the nucleotide sequence of the *eibG*-like gene, called *eibH*, are HM114306, HM114307, HM114308 and HM114309.

Genotypic serotyping

No EPEC or VTEC isolate was found to be positive with the PCRs for the tested serogroups (O157, O26, O111, O103 and O145).

Antibiotic susceptibility tests

Two isolates (both VTEC) were sensitive to all the tested antibiotics, and only 10·8% of the isolates (two EPEC and two VTEC) were intermediate or resistant to more than three of the eight antibiotics (Table 4 and Fig. 1).

All 37 EPEC and VTEC isolates were sensitive to the association of trimethoprim–sulfamethoxazole to enrofloxacin and to gentamicin, but 24·3, 21·6 and 8·1% were, respectively, intermediate to florfenicol, to tetracycline and to ceftiofur. Conversely 81·1% of isolates were intermediate or resistant to the association of amoxicillin–clavulanic acid, and 35·1% were resistant to spectinomycin.

Table 4 Results obtained for each strain for the antibiotic resistance pattern

Species	Animal no.	Strain no.	Antibiotic resistance pattern
Capreolus capreolus	A09/305	A09/305.1	AMC, FF, TTC: I
		A09/305.2	AMC, C: I
	A09/311	A09/311.1	AMC, FF, TTC: I
	A09/325	A09/325.1	AMC: I
		A09/325.2	AMC: I
	A09/332	A09/332.1	FF: I
	A09/335	A09/335.1	AMC: I
	A09/337	A09/337.1	AMC: I
		A09/337.2	AMC: I
	A09/378	A09/378.1	AMC: I
		A09/378.2	FF: I
	CH09/03	CH09/03.1	AMC: I
	CH09/90	CH09/90.1	AMC: I; Sp: R
		CH09/90.2	AMC, FF, TTC: I
		CH09/90.3	AMC, C, TTC: I; Sp: R
Cervus elaphus	A09/299	A09/299.1	AMC: I
		A09/299.2	AMC: I
		A09/299.3	AMC: I
	A09/313	A09/313.1	AMC: I
		A09/313.2	/
	A09/315	A09/315.1	AMC, FF: I; Sp: R
		A09/315.2	AMC, FF, TTC: I; Sp: R
	A09/340	A09/340.1	AMC, C: I
		A09/340.2	AMC, TTC: I
		A09/340.3	AMC, TTC: I
	A09/345	A09/345.1	AMC, FF, TTC: I; Sp: R
		A09/345.2	AMC, FF, TTC: I; Sp: R
	A09/347	A09/347.1	AMC: I
	A09/371	A09/371.1	AMC: I; Sp: R
		A09/371.2	AMC: I; Sp: R
		A09/371.3	Sp: R
	A09/386	A09/386.1	AMC: I; Sp: R
	A10/42	A10/42.1	/
	C09/73	C09/73.1	Sp: R
	C09/149	C09/149.1	AMC, Sp: R
		C09/149.2	AMC: I
		C09/149.3	Sp: R

AMC, amoxicillin/acid clavulanic; C, ceftiofur; FF, florfenicol; Sp, spectinomycin; TTC, tetracycline; R, resistant; and I, intermediate.

Discussion

Because wild ruminants can represent a source of EHEC or VTEC infection for humans, and possibly also for domestic ruminants (Pierard *et al.* 1997; Simpson 2002; Miko *et al.* 2009), the purpose of this study was to determine the prevalence of EHEC, VTEC and EPEC strains in free-ranging wild ruminants in Belgium during two consecutive hunting seasons. Unlike previous studies (Fischer *et al.* 2001; Renter *et al.* 2001; Kemper *et al.* 2006; Heuvelink *et al.* 2008), we did not focus on the O157:H7 EHEC serotype. The overall prevalence rate of healthy carriers of

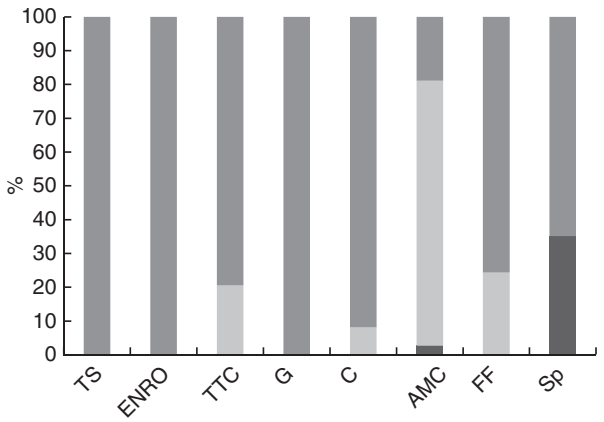

Figure 1 Representation of the percentage of sensitive, intermediate and resistant strains to the tested antibiotics. AMC, amoxicillin/acid clavulanic; C, ceftiofur; ENRO, enrofloxacin; FF, florfenicol; G, gentamicin; Sp, spectinomycin; TS, trimethoprim/sulfamethoxasol; and TTC, tetracycline. (■) Sensitive; (□) intermediate and (■) resistant.

EPEC, VTEC and EHEC strains among wild cervids in the south of Belgium was found to be 15%, or 20 of 133 animals. Of these 20 animals, four were carriers of EPEC, 15 of VTEC and one animal a carrier of EPEC and VTEC; none of the animals was found to carry an EHEC strain. To our knowledge, this is the first such broad study in Belgium, and only a few such studies have been performed worldwide (Asakura *et al.* 1998; Fukuyama *et al.* 1999; Sanchez *et al.* 2009).

In 2009, Sánchez *et al.* found a prevalence of 23·9% of VTEC *sensu stricto* strains in wild ruminants in Spain. This study as well as ours suggested that the prevalence of VTEC and EPEC strains in wild ruminants can be high. Moreover, our technical approach (picking three lactose-fermenting colonies for each faecal sample) lacks sensitivity, and the 'real' prevalence is probably higher. Therefore, both studies suggest that prevalence studies should not focus solely on O157:H7 EHEC (Fischer *et al.* 2001; Renter *et al.* 2001; Kemper *et al.* 2006; Heuvelink *et al.* 2008). Such restricted studies are interesting in demonstrating a potential source of O157:H7 EHEC infection for humans. However, they tend to underestimate the potential risk of transmission of other VTEC, EHEC and EPEC strains from wild ruminants to humans via the consumption of venison and to domestic ruminants owing to the sharing of the same pasture. Indeed, many serogroups other than O157 EHEC have also been shown to be responsible for outbreaks in humans in several countries (Bettelheim 2007; Miyajima *et al.* 2007), and about one quarter of confirmed EHEC/VTEC cases in humans are caused by untyped or untypeable strains (Anonymous 2010). In some countries, strains that do not belong to the 'gang of five' (O157, O26, O111, O103 and O145) have been shown to be responsible for a high proportion of human cases (e.g. 66% in Germany and 44% in Sweden) (Anonymous 2010). It is also worth noting that 11 (69%) of the wild cervids in the present study were found to carry VTEC strains that are PCR positive for a gene encoding one Stx2 toxin, of which two variants (Stx2 and Stx2c) are the most potent in humans and are frequently responsible for HUS (Bonnet *et al.* 1998).

The other typing results of the 31 VTEC isolates were negative for the *subA* and *saa* gene (as was demonstrated in (Sanchez *et al.* 2009) for the *saa* gene), and only one isolate was positive for the *EHEC-hlyA* (unlike in (Sanchez *et al.* 2009). However, eight isolates from four adult cervids (A09/337, A09/378, A09/313 and C09/149) were found here to harbour an *eibG-like* gene, called *eibH*, which is 88% homologous to the published *eibG* gene sequence (AB255744). To date, the *eibG* gene has only been found in human VTEC *sensu stricto* strains (Lu *et al.* 2006). This new variant is perhaps specific to wild and/or domestic ruminants. To confirm this hypothesis, the distribution of the *eibG-like* gene needs to be studied in other VTEC strains from wild and domestic ruminants, from humans and from other potential reservoirs (wild and domestic pigs, birds, cats, dogs, etc.).

All the five EPEC isolates identified in the present study are 'atypical EPEC' or a-EPEC, because they do not harbour any *bfpA* genes. This is in full agreement with the observation that Bfp is only produced in human strains, with the exception of a few strains from dogs and cats (Goffaux *et al.* 2000; Chen and Frankel 2005). Nevertheless, current studies suggest that a-EPEC strains are emerging pathogens that are becoming even more frequent than the typical EPEC strains in humans in both developing and developed countries (Hernandes *et al.* 2009). If this is confirmed in the future, we cannot exclude animal EPEC strains as potential zoonotic pathogens.

As expected, the results of the antibiotic sensitivity testing of the 37 VTEC and EPEC isolates showed a much lower sensitivity in comparison with *E. coli* isolates from cattle (Martin *et al.* 2007; Srinivasan *et al.* 2007). Indeed, in the present study, only 21·6% of the isolates isolated from wild ruminants were found to be resistant (intermediate or resistant) to more than two antibiotics. Moreover, these isolates did not show a strong resistance to the antibiotics; most of them were intermediate to the antibiotics. Additionally, some *E. coli* isolated from the same animal presented different antibiotic susceptibility profiles. This could be explained by the fact that these isolates (intermediate or resistant) are at the limit of being sensitive. The fact that wild animals are normally not in close contact with antibiotics could explain their low level of resistance (Thaller *et al.* 2010). The remaining

antibiotic resistance level could be explained by: (i) the exposure to antibiotics or related antimicrobial chemicals in their food intake (through complementary food in winter); (ii) the transmission of resistant strains from cattle to wild ruminants because of their close contact; and/or (iii) the persistence of antibiotics in the environment.

More similar studies need to be performed on wild and farmed cervids in different countries, and future research should also focus on: (i) collecting more data on carrier prevalence of non-O157 EHEC, of VTEC and of a-EPEC; (ii) evaluating the pathogenic potential of these strains in wild cervids, if any; (iii) analysing the risk of transmission to humans and to domestic ruminants; but also (iv) studying the possible contamination of wild cervids by domestic ruminants.

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