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Verocytotoxin-producing *Escherichia coli* in Chamois (*Rupicapra rupicapra*) and Cattle in Austria

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ABSTRACT: We assessed the prevalence of verotoxigenic *Escherichia coli* (VTEC) in chamois (*Rupicapra rupicapra*) and livestock grazing on a mountain pasture in Austria during June–August 2009. We detected VTEC throughout the sampling period in high numbers in cattle as well as in chamois, leading to the assumption that the degree of contamination of the environment is high. This is the first report of pathogenic *E. coli* identified in chamois, implicating chamois as a new potential reservoir of these zoonotic pathogens. Because the study area also serves recreational purposes, there is a risk of humans acquiring infection via direct or indirect contact.

Key words: Cattle, chamois, enteropathogenic *Escherichia coli*, prevalence, *Rupicapra rupicapra*, verotoxigenic *Escherichia coli*.

Pathogenic *Escherichia coli* are known to cause urinary tract infection, sepsis, meningitis, and enteric or diarrheal disease in humans. Verotoxigenic, enterohemorrhagic, and enteropathogenic *E. coli* (VTEC, EHEC, and EPEC, respectively) are important representatives of the diarrheagenic group (Nataro and Kaper, 1998). Verotoxigenic *E. coli* (also known as shiga toxin-producing *E. coli* [STEC]) carry *stx1* and/ or *stx*2 genes that encode for two types of verotoxins (shigatoxins) and that are predominantly associated with diarrhea in piglets and humans (Mainil and Daube, 2005). Enteropathogenic *E. coli* carry the *eae*-gene encoding for intimin, which causes attaching and effacing lesions on enterocytes. Similarly, the *eae*-gene can be detected in EHEC, which additionally carry *stx*-genes. The EHEC represent a subset of serotypes of VTEC that are tightly linked to severe human illnesses, including hemorrhagic colitis and hemolytic uremic syndrome in young children (Nataro and Kaper, 1998). In contrast, ruminants are known to be asymptomatic carriers of EHEC, with serotype O157:H7 being the most reported and best studied representative (Caprioli et al., 2005). Several studies stressed the link between human infection with *E. coli* O157:H7 and its ruminant origin (Mainil and Daube, 2005). Besides EHEC infection via contaminated food or direct contact with shedders, previous work highlighted the increasing occurrence of environmental transmission (Grif et al., 2005; Muniesa et al., 2006). Therefore, further investigation of the role of wild ruminants as VTEC reservoirs is recommended (Sánchez et al., 2009). In Austria, there are limited data on the carrier status of cattle and no data on wild ruminants.

We conducted a survey of the prevalence of pathogenic *E. coli* in cattle and chamois (*Rupicapra rupicapra*) on an alpine pasture in the Northern Limestone Alps to investigate possible contamination of the mountain environment. We studied 53 cattle (from nine farms) and several free-ranging chamois that shared a common pasture during the summer. Five samplings were conducted every 2–3 wk from June to August 2009. Fresh fecal specimens from cattle ($n=217$) were collected into sterile containers immediately after defecation. All samples were individually assignable by ear tags that allowed us to monitor the temporal changes in EHEC infection in each animal over the sampling period. From the third sampling onward, three to 13 chamois samples (total $n=24$) were included in the survey. The condition of the fecal pellets from chamois ranged from dry to fresh. To verify the origin of the collected droppings, photographs were taken and exam-

TABLE 1. Primers used to amplify fragments of genes specific for pathogenic *Escherichia coli* from livestock and chamois (*Rupicapra rupicapra*) on a mountain pasture in Austria, June–August 2009.

Target gene	Oligonucleotide sequence (5′-3′)	Size of amplified product (base pairs)	Reference
rfbE	AAGATTGCGCTGAAGCCTTTG CATTGGCATCGTGTGGACAG	497	Fortin et al. (2001)
eae	TCAATGCAGTTCCGTTATCAGTT GTAAAGTCCGTTACCCCAACCTG	482	Vidal et al. (2004)
stx1	CAGTTAATGTGGTGGCGAAGG CACCAGACAATGTAACCGCTG	348	Cebula et al. (1995)
stx2	ATCCTATTCCCGGGAGTTTACG GCGTCATCGTATACACAGGAGC	584	Cebula et al. (1995)

ined by a wildlife expert. We were careful to avoid sample contamination with soil. After collection, the samples were stored at 4 C until further processing (≤24 hr). Specimens (25 g each) were enriched in 225 ml of buffered peptone water (Merck, Darmstadt, Germany) at 37 C for 19 hr without agitation. DNA was extracted using the FastDNA® Spin Kit for Soil (MP Biomedicals, Eschwege, Germany). Samples were analyzed using four PCR targeting the virulence genes *stx1*, *stx2*, *eae*, and *rfbE*, a gene specific for *E. coli* O157:H7 (Table 1). The PCRs were carried out according to previously described protocols (Cebula et al., 1995; Fortin et al., 2001; Vidal et al., 2004). The PCR products were examined by standard agarose gel (1%) electrophoresis.

Before the cattle were driven onto the pasture, we randomly screened 12 cows (at least one cow per farm). Nine cows (75%), from seven (78%) of nine farms, were PCR-positive for at least one virulence gene (Table 2). Eight of these nine cows (89%) were positive for VTEC and one cow (11%) for EPEC. In the second sampling, 4 days later, 38 of 46 (83%) cattle were positive; 37 (97%) presumably were VTEC carriers and one (3%) was an EPEC carrier. In the third, fourth, and fifth samplings, the prevalences in cattle were 81% (43/53), 70% (37/53), and 74% (39/53), respectively. Of the 43 positive specimens on the third sampling, 41 (95%) were positive for VTEC and two (5%) for EPEC strains. On the fourth sampling, 34 samples (92%) showed VTEC and three (8%) EPEC gene sequences. In the last sampling, 27 (69%) samples were VTEC-positive and 12 (31%) were EPEC-positive. A mean of 20.3% of the samples (44/217) exhibited a positive signal for the *rfbE*-gene over the sampling period, whereas 9.2% (20/217) had evidence for strains of EHEC serogroup O157 (Fortin et al., 2001; Table 2).

The collection of samples from chamois began with the third sampling, and two of three fecal specimens were positive for VTEC-gene *stx1*. In the fourth sampling, eight of eight were VTEC-positive. In the fifth sampling, eight of 13 (62%) were positive for VTEC, and three of 13 (23%) were EPEC-positive. However, none of the chamois carried the *rfbE*-gene, indicating the presence of EHEC O157.

To our knowledge this is the first confirmed finding of VTEC in Chamois. Serogroup O157 was absent from Chamois; however, because cattle and chamois shared the same pasture, interspecific transmission can be assumed and it is likely that O157-positive chamois will be found in the near future (Leotta et al., 2006). To draw epidemiologic conclusions and confirm this assumption, we will use serotyping and molecular typing methods (e.g., pulsed field gel electrophoresis or ribotyping) in future studies. Because pathogenicity of VTEC correlates with the presence of various virulence genes

TABLE 2. Prevalence of various *Escherichia coli* gene combinations (*stx1*, *stx2*, *eae*, *rfbE*) detected in fecal specimens of chamois (*Rupicapra rupicapra*) and livestock grazing on a mountain pasture in Austria, June–August 2009.

Group	No. positive cattle related to detected gene combination[a]					Combination of genes				No. positive chamois related to detected gene combination[a]					Combination of genes			
	A (12)	B (46)	C (53)	D (53)	E (53)	*rfbE*[b]	*eae*	*stx1*	*stx2*	X	X	C (3)	D (8)	E (13)	*rfbE*[b]	*eae*	*stx1*	*stx2*
1	1	3	3	1	1	+[c]	+	−[d]	+									
2[e]	3	8	10	16	14	−	−	−	−			1	–	2	–	–	–	–
3[e]	2	5	6	2	1	−	+	+	+			–	2	2	–	+	+	+
4[e,f]	1	1	2	3	12	−	+	−	−			–	–	3	–	+	−	−
5	1	–	1	–	–	+	−	+	+									
6	1	–	1	7	6	+	−	−	−									
7[e]	1	8	13	5	3	−	−	−	+			–	–	1	–	–	–	+
8	1	1	–	–	2	+	+	−	−									
9[e]	1	11	6	5	5	−	+	−	+			–	1	–	–	+	−	+
10[e]	–	3	–	2	3	−	+	+	−			–	–	2	–	+	+	−
11[e]	–	2	6	3	1	−	−	+	+			–	–	1	–	−	+	+
12[e]	–	1	4	3	1	−	−	+	−			2	5	2	–	−	+	−
13	–	2	–	3	2	+	−	−	+									
14	–	1	1	–	1	+	+	+	+									
15	–	–	–	3	1	+	+	+	−									
Total positives (%)	9 (75)	38 (83)	43 (81)	37 (70)	3 9 (74)							2(67)	8(100)	11(85)				

[a] Samplings periods: A = 12 June 2009, B = 17 June 2009; C = 1 July 2009; D = 21 July 2009; E = 12 August 2009; X = no animals sampled.

[b] *Escherichia coli* O157-specific gene.

[c] + = gene detectable.

[d] − = gene/gene combination not detectable.

[e] Gene combinations also found in chamois.

[f] Enteropathogenic *E. coli*.

and their combinations, we used PCR for screening. From a public health perspective, Shiga toxins are considered the most important virulence factors (Leotta et al., 2006). However, in combination with the colonizing factor *eae*, pathogenicity of STEC and therefore severity of disease in humans increases further (Beutin, 2006).

Given the large proportion of VTEC- (75%) and EPEC-positive (12.5%) chamois in this small sample, we assume that the overall prevalence of pathogenic *E. coli* in chamois in this area is high. Because the home range of chamois exceeds that of cattle (and also considering likely interspecific transmission), chamois may contribute to further spread of acquired VTEC strains in the mountain environment. Fremaux et al. (2010) showed that wildlife in alpine regions is a possible source of water and environmental contamination with VTEC. This can have important public health implications in terms of consumption of contaminated raw milk and other dairy products (obtained from infected dairy cattle grazing on alpine pastures), which are often sold in mountain shelters. Similarly, cattle could spread strains acquired from wild animals in the farm environment on return from alpine pastures thereby presenting infection risk to humans.

Our results do not reveal whether the positive animals were infected with one strain bearing all four genes or whether they hosted more strains carrying various combinations of virulence genes (Table 2). However, the distinction between VTEC, EPEC, and EHEC serotype O157 carriers is possible. Despite *E. coli* O157:H7 being a highly pathogenic serotype commonly detected in seriously ill humans, non-O157 serotypes are also frequently isolated from hemorrhagic colitis and hemolytic uremic syndrome patients (Muniesa et al., 2006). Knowledge concerning the occurrence of non-O157 serotypes in animal reservoirs is limited and deserves further attention (Sánchez et al., 2009).

Grazing cattle on alpine pastures is common practice in Austria. Given the established prevalence of pathogenic *E. coli* and the use of the study site for recreation, there is a risk of human infection. People, especially children, were frequently observed petting and feeding cattle. Fecal shedding of EHEC occurs intermittently and transiently with peaks in the warmer months. Therefore, direct or indirect contact may pose a risk of infection to recreational users, particularly children, in this area (Heuvelink et al., 2002). The low infectious dose (1–10 colony-forming units), absence of hygienic measures during alpine recreation, and the ability of EHEC to survive for up to 150 days in the environment also favor infection (Maule, 2000; Fortin et al., 2001). In addition, heavy rainfall is associated with bacterial contamination of water, possibly putting public and rural water supplies at risk (Thomas et al., 2006). Synge (2000) discussed strategies for minimizing pathogen shedding in cattle feces to reduce environmental contamination and the likelihood of human infection. Nevertheless, all approaches proved to be impractical or requiring additional research.

In conclusion, we showed that VTEC was prevalent during the entire sampling period in both cattle and chamois. Because this is the first report of the isolation of VTEC from chamois, further studies should be undertaken to fully understand the role of chamois as a potential reservoir. Moreover, given the zoonotic potential, it is essential also to consider non-O157 strains in future epidemiologic surveys in animals. Standardizing detection methods and surveillance in all countries and creating public awareness of risk factors are also important to minimize human infection.

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