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Occurrence of verocytotoxin-producing *Escherichia coli* in the faeces of free-ranging wild lagomorphs in southwest Spain

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Abstract Verocytotoxin-producing *Escherichia coli* (VTEC) is an important group of emerging food-borne pathogens and represents a major public health concern worldwide. The aim of this work was to analyse faecal samples from hunted wild lagomorphs for the presence of *E. coli* O157:H7 and non-O157 VTEC. During two hunting seasons, faecal samples from 241 animals were collected, including wild rabbit (*Oryctolagus cuniculus*) and hare (*Lepus granatensis*) and were examined for VTEC. Overall, VTEC were detected and

isolated in four (1.66%) of the 241 animals sampled. *E. coli* O157:H7 was isolated only from one of 124 (0.81%) wild rabbit faecal samples while non-O157 VTEC were isolated from two of 124 (1.61%) wild rabbit faecal samples and one of 117 (0.85%) hare faecal samples. VTEC isolates obtained in the present study (four in total) belonged to four different O:H serotypes, including two serotypes (O84:H– and O157:H7) previously associated with human infection and in particular with causing the life-threatening haemolytic–uraemic syndrome. Although these results indicate a low prevalence of VTEC infection in free-ranging wild lagomorphs, they may play an important role as a source of exposure to human beings and livestock and as a vehicle for dispersing these pathogens. These findings have implications for the zoonotic risk to hunters, people consuming meat from wild animals and others in contact with wild animal faeces, and also in the development of programmes for controlling VTEC at the farm level.

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Verocytotoxin-producing *Escherichia coli* (VTEC) has recently emerged as an important food-borne pathogen. Human diseases ranging from mild diarrhoea to haemorrhagic colitis and the life-threatening haemolytic-uraemic syndrome (HUS) can be caused by VTEC, typically affecting infants, young children and the elderly (Paton and Paton 1998). The pathogenic capacity of VTEC resides in a number of virulence factors, including verocytotoxins (VT1 and VT2), intimin, enterohaemolysin and the autoagglutinating adhesin (Saa) (Gyles 2007). Serotype O157:H7 especially represents a major public health concern worldwide; however, non-O157 VTEC should not be overlooked

in human disease investigations because non-O157 VTEC strains are more prevalent than *E. coli* O157:H7 in the faeces of meat-producing animals, indicating that humans are more likely to become exposed to such strains (Blanco et al. 2004a; Djordjevic et al. 2004). Although healthy domestic ruminants (mainly cattle) are the best recognised animal reservoir for VTEC (Blanco et al. 2004b; Sánchez et al. 2010a), VTEC strains have also been isolated from wildlife, especially from deer (Renter et al. 2001; Sánchez et al. 2009). The isolation of *E. coli* O157:H7 and non-O157 VTEC from wild rabbits has been previously reported (Pritchard et al. 2001; Scaife et al. 2006) and colonisation of laboratory rabbits by VTEC has also been demonstrated in experimental laboratory infections (Li et al. 1993; García and Fox 2003). The aim of the current study was to analyse faecal samples from wild rabbits and hares killed by hunters and intended for their own consumption for the presence of *E. coli* O157:H7 and non-O157 VTEC.

During the 2007–2008 and 2008–2009 hunting seasons (from October to January), collaborating hunters collected faecal samples from wild lagomorphs harvested in different game estates in the Extremadura region in southwest Spain. They were provided with sampling equipment as well as step-by-step instructions for sample collection before the start of every season. When harvesting the animal, the hunters were asked to use a pair of disposable gloves, insert a sterile swab into the rectum of the animal, collect a faecal sample (one per animal) by rapid in-and-out motion and transfer the swab to a tube containing Cary-Blair transport medium (DeltaLab). The samples were transported to the laboratory under refrigeration and placed in culture media within 24 h. Overall, wild rabbit (*Oryctolagus cuniculus*) provided 124 samples and 117 came from hare (*Lepus granatensis*). The samples were plated onto both MacConkey (MAC) and cefixime tellurite sorbitol MacConkey (CTSMAC) agars (Oxoid). For isolation of non-O157 VTEC, ten suspect *E. coli* colonies from MAC plates were tested for the genes encoding VT1 and VT2 toxins (VT1 and VT2 genes) by PCR as previously described (Rey et al. 2003). For isolation of *E. coli* O157:H7, after a selective enrichment

step involving immunomagnetic separation, ten non-sorbitol-fermenting colonies from the CTSMAC plates were tested for the genes encoding O157 and H7 antigens as previously described (García-Sánchez et al. 2007). The resulting VTEC isolates were confirmed biochemically as *E. coli* by the API 20E system (bioMérieux) and tested for additional virulence genes (*eae*, *ehxA* and *saa*). The identification of O and H antigens (serotyping) in VTEC isolates was carried out as described by Guinée et al. (1981) using the full range of O (O1 to O185) and H (H1 to H56) antisera.

Overall, VTEC were detected and isolated in four (1.66%) of the 241 animals sampled. *E. coli* O157:H7 was isolated only from one of 124 (0.81%) wild rabbit faecal samples while non-O157 VTEC were isolated from two of 124 (1.61%) wild rabbit faecal samples and one of 117 (0.85%) hare faecal samples (Table 1). All isolates obtained in the present study (four in total) were further characterised (Table 1). They belonged to four different O:H serotypes, including two serotypes (O84:H– and O157:H7) previously associated with human infection and in particular with causing HUS (<http://www.lugo.usc.es/ecoli>; <http://www.microbionet.com.au/vtactable.htm>). The PCR procedure indicated that one isolate carried the VT1 gene and the rest carried the VT2 gene. The *ehxA* and *eae* genes were detected only in the *E. coli* O157:H7 isolate and none of the isolates contained the *saa* gene.

The results of the present study suggest a low prevalence of VTEC infection in free-ranging wild lagomorphs in this area of Spain, although it is important to note that this was not a random survey but only biased to areas used by hunters. Prevalence rates of *E. coli* O157:H7 and non-O157 VTEC obtained in this study are much lower than those currently observed in domestic ruminant species such as cattle, sheep or goats (Oporto et al. 2008; Orden et al. 2008; Sánchez et al. 2010a). This is not surprising as livestock are much more intensively reared and the potential for exposure and colonisation is much greater than for most wildlife; however, VTEC strains have also been isolated from other wild animal species, such as deer and wild boars (Sánchez et al. 2009, 2010b). Wild rabbits and hares are

Table 1 Results of faecal culture for VTEC, serotypes and virulence genes of VTEC isolates recovered from wild lagomorphs

Animal	Number cultured	<i>E. coli</i> O157:H7		Non-O157 VTEC		
		Number positive (%)	Virulence genes	Number positive (%)	Serotype	Virulence genes
Wild rabbit	124	1 (0.81)	VT2 <i>eae ehxA</i>	2 (1.61)	O22:HNT O117:H8/H21 ^a	VT2
Hare	117	0		1 (0.85)	O84:H ^{-b}	VT1

VT1/VT2 genes encoding VT1 and VT2 toxins, *eae* gene encoding intimin, *ehxA* gene encoding enterohaemolysin, HNT H antigen non-typeable

^a The isolate cross-reacted with the respective H antisera

^b Serotype previously associated with human infection and in particular with causing HUS

commonly found in areas inhabited by human beings and livestock and consequently may share common sources of exposure to VTEC. Indeed, wild rabbits' faeces have been associated with *E. coli* O157:H7 infection in visitors to a wildlife park in the United Kingdom (Pritchard et al. 2001). Furthermore, indistinguishable genotypes of *E. coli* O157:H7 have been identified (by the pulsed-field gel electrophoresis technique) in cattle and wild rabbits occupying the same range, indicating either a common source of exposure or transmission from one species to the other (Pritchard et al. 2001). In the studied areas, wild rabbit and hare populations usually have access to the same pastures as cattle and sheep, although they have no contact with free-ranging wild ruminants. Therefore, environmental contamination with VTEC excreted by domestic livestock and indirect transmission to wild lagomorphs from the contaminated environment could explain the occurrence of VTEC in their faeces. In addition, Miko et al. (2009) recently reported the contamination of meat from wild animals (including hare meat) with VTEC and concluded that such strains should be recognised as a public health problem. Consequently, wild lagomorphs such as wild rabbits and hares may play an important role as a source of VTEC in the environment, as a source of exposure to human beings and livestock and as a vehicle for dispersing these pathogens. These findings have implications for the zoonotic risk to hunters, people consuming meat from wild animals and others in contact with wild animal faeces, and also in the development of programmes for controlling VTEC at the farm level.

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