



## Short communication

Detection and characterisation of O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli* in wild boars

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## ABSTRACT

The aim of this work was to determine the prevalence and characteristics of *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* (STEC) in free-ranging wild boars killed during the hunting season in southwest Spain. Faecal samples from 212 wild boars (*Sus scrofa*) were collected and examined for STEC. Characterisation of isolates was performed by PCR, serotyping, phage typing, and pulsed-field gel electrophoresis (PFGE). *E. coli* O157:H7 and non-O157 STEC were isolated from 7 (3.3%) and 11 (5.2%) animals, respectively, and the resulting 19 isolates were characterised. The PCR procedure indicated that 4 isolates carried the *stx*<sub>1</sub> gene, 12 carried the *stx*<sub>2</sub> gene, and 1 contained both of these genes. The *ehxA*, *eae*, and *saa* genes were detected in 13, 8, and 1 of the isolates, respectively. The *eae*-positive isolates comprised the types *eae*-γ1 and *eae*-ζ. The isolates belonged to 11 O:H serotypes, including 4 new serotypes not previously reported within STEC strains, and the majority of them were from serotypes previously associated with human infection. *E. coli* O157:H7 isolates belonged to phage types associated with severe human illness: PT14, PT34, and PT54. Indistinguishable PFGE types were found in *E. coli* O157:H7 isolates recovered from a wild boar and from a human patient with diarrhoea living in the same geographic area.

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## 1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) have recently emerged as important food-borne pathogens, especially serotype O157:H7. Human diseases ranging from mild diarrhoea to haemorrhagic colitis and haemolytic uraemic syndrome (HUS) can be caused by STEC,

typically affecting children, the elderly, and immunocompromised patients (Centers for Disease Control and Prevention, 2001). The pathogenic capacity of STEC resides in a number of virulence factors, including Shiga toxins (Stx1 and Stx2), intimin, enterohaemolysin, and the STEC autoagglutinating adhesin (Saa) (Gyles, 2007).

Healthy domestic ruminants such as cattle, sheep, and goats can harbour STEC and *E. coli* O157:H7 in their faeces and are thus natural reservoirs of these pathogens (Blanco et al., 2004b; Orden et al., 2008; Sánchez et al., 2009b). However, *E. coli* O157:H7 and non-O157 STEC strains have also been isolated from wild ruminants (Renter et al., 2001; García-Sánchez et al., 2007; Sánchez et al., 2009a), and deer

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have been implicated in the food-borne transmission of *E. coli* O157:H7 to humans in Japan (Nagano et al., 2004) and in the United States (Keene et al., 1997; Rabatsky-Ehr et al., 2002). Recent experimental and epidemiologic studies suggest that domestic pigs are biologically competent hosts and a potential reservoir of *E. coli* O157:H7 (Feder et al., 2003; Cornick and Helgerson, 2004). Actually, *E. coli* O157:H7 strains have been identified in wild boars in Sweden (Wahlström et al., 2003) and feral swine in the United States, where feral swine near spinach fields shed the outbreak strain in a nationwide spinach-associated outbreak of *E. coli* O157:H7 (Jay et al., 2007). Nevertheless, there are no reports of the isolation of STEC other than *E. coli* O157:H7 from wild boars and, to our knowledge, the occurrence of *E. coli* O157:H7 and non-O157 STEC in wild boars in Spain is not known. The objectives of this study were to establish the prevalence of *E. coli* O157:H7 and non-O157 STEC in free-ranging wild boars killed during the hunting season in southwest Spain and to characterise them, in order to determine if wild boars represent a potential risk to public health.

## 2. Materials and methods

### 2.1. Sample collection, culture, and STEC screening

During the 2007–2008 hunting season (from mid-October to mid-February), a total of 212 European wild boars (*Sus scrofa*) killed by hunters in different game estates in the Extremadura region in southwest Spain were sampled. On each sampling occasion, one sample of rectal faeces per animal was collected from harvested individuals. Samples were transported to the laboratory under refrigeration in Cary-Blair transport medium (Deltalab, Barcelona, Spain) and placed in culture media within 24 h of collection.

For isolation of non-O157 STEC, faecal samples were plated directly onto MacConkey agar (Oxoid, Basingstoke, England). Following overnight incubation, 10 suspect *E. coli* colonies were tested for the genes encoding Stx1 and Stx2 toxins (*stx*<sub>1</sub> and *stx*<sub>2</sub> genes) by PCR as previously described (Rey et al., 2003). The resulting STEC isolates were confirmed biochemically as *E. coli* by the API 20E system (bioMérieux, Marcy L'Etoile, France) and tested for the genes encoding intimin (*eae* gene), enterohaemolysin (*ehxA* gene), and Saa (*saa* gene) as previously described (Sánchez et al., 2009a). When isolates from a given sample exhibited similar genetic characteristics in terms of the presence or absence of virulence genes, only one colony was selected and stored at –80 °C until further characterisation. Otherwise, when isolates with different genetic characteristics were obtained, one colony of each was selected and stored for further characterisation. For isolation of *E. coli* O157:H7, faecal samples were examined by enrichment culture in modified buffered peptone water, concentration of *E. coli* O157 by immunomagnetic separation with Dynabeads anti-*E. coli* O157 (Dyna, Oslo, Norway), and culture of magnetic beads onto cefixime tellurite sorbitol MacConkey agar (Oxoid). Following overnight incubation, 10 non-sorbitol-fermenting colonies were tested for the genes encoding O157 and H7 antigens

(O157 *rfbE* and *fliCh7* genes) by PCR as previously described (García-Sánchez et al., 2007). The resulting *E. coli* O157:H7 isolates were confirmed biochemically as *E. coli* by the API 20E system (bioMérieux) and tested for the genes encoding Stx1 and Stx2 toxins, intimin (*eae* and *eae*- $\gamma$ 1 variant), and enterohaemolysin.  $\beta$ -Glucuronidase (GUD) activity in *E. coli* O157:H7 isolates was investigated on Chromocult Coliform agar (Merck, Darmstadt, Germany) after overnight incubation. The *eae* type was determined for all *eae*-positive isolates as previously described (Rey et al., 2003).

### 2.2. Characterisation of isolates

The identification of O and H antigens in isolates was carried out as described by Guinée et al. (1981) using the full range of O (O1–O185) and H (H1–H56) antisera. All antisera were absorbed with corresponding cross-reacting antigens to remove non-specific agglutinins. The O antisera were produced in the Laboratorio de Referencia de *E. coli* (Lugo, Spain) and the H antisera were obtained from the Statens Serum Institut (Copenhagen, Denmark).

The phage typing of *E. coli* O157:H7 isolates was performed as described by Khakhria et al. (1990) using phages provided by the National Microbiology Laboratory (Public Health Agency of Canada, Winnipeg, Canada).

Pulsed-field gel electrophoresis (PFGE) was used to establish relatedness and diversity among *E. coli* O157:H7 isolates. PFGE was performed in accordance with the PulseNet-Europe protocol (<http://www.pulsenet-europe.org/docs.htm>). Genomic DNA was digested with XbaI (Roche Diagnostics, Mannheim, Germany) and analyzed in 1% agarose gels (Bio-Rad, Hemel Hempstead, United Kingdom) in 0.5 $\times$  TBE buffer at 14 °C using the CHEF MAPPER system (Bio-Rad). The runtime was 21.3 h at 6 V/cm, with initial and final switch times of 2.16 and 54.17 s, respectively. The XbaI-digested DNA from *Salmonella enterica* Braenderup H9812 was used as a molecular size marker. Resultant images were analyzed with the InfoQuestFP software (Bio-Rad). Isolates were allocated a different PFGE type when a genetic difference could be detected. Cluster analysis was performed using the Dice coefficient and the unweighted pair group method with arithmetic averages (UPGMA).

## 3. Results and discussion

Overall, STEC were detected in 17 (8.0%) of the animals sampled. *E. coli* O157:H7 was isolated from 7 (3.3%) animals and non-O157 STEC were isolated from 11 (5.2%). Two different strains were identified in samples from 2 animals, in one of which both *E. coli* O157:H7 and non-O157 STEC (O6:H10) were isolated. These results indicate a high prevalence of STEC infection in free-ranging wild boars in this area of Spain, with a higher prevalence rate of *E. coli* O157:H7 (3.3%) than in a previous study in Sweden (1.5%) (Wahlström et al., 2003).

All isolates obtained in the present study (19 in total) were further characterised (Table 1). They belonged to 9 O serogroups and 11 O:H serotypes, including 4 new serotypes not previously reported within STEC strains.

**Table 1**

Association between serotype and virulence genes for isolates recovered from wild boars.

Serotype <sup>a</sup>	No. of isolates	Virulence genes profile <sup>b</sup>				
		<i>stx</i> <sub>1</sub>	<i>stx</i> <sub>2</sub>	<i>eae</i>	<i>ehxA</i>	<i>saa</i>
O6:H10	1	+				
O23:H21 <sup>c</sup>	2		+			
O104/O127:H– <sup>d,e</sup>	1		+		+	
O104/O127:H1/H12 <sup>c,e</sup>	1	+				+
O109:H–	1	+		+ (ζ)	+	
O127:H2 <sup>c</sup>	1		+		+	
O142:H8/H21 <sup>c,e</sup>	1	+				
O146:H21 <sup>f</sup>	1	+	+		+	
O157:H7 <sup>d</sup>	2			+ (γ1)	+	
O157:H7 <sup>d</sup>	5		+	+ (γ1)	+	
O157:H21	2		+		+	
ONT:H7 <sup>f</sup>	1		+			

<sup>a</sup> ONT, O antigen non-typeable.

<sup>b</sup> +, gene detected by PCR.

<sup>c</sup> Serotype not previously reported within STEC strains.

<sup>d</sup> Serotype previously associated with human STEC strains that have caused HUS.

<sup>e</sup> Each of the isolates cross-reacted with the respective O and H antisera.

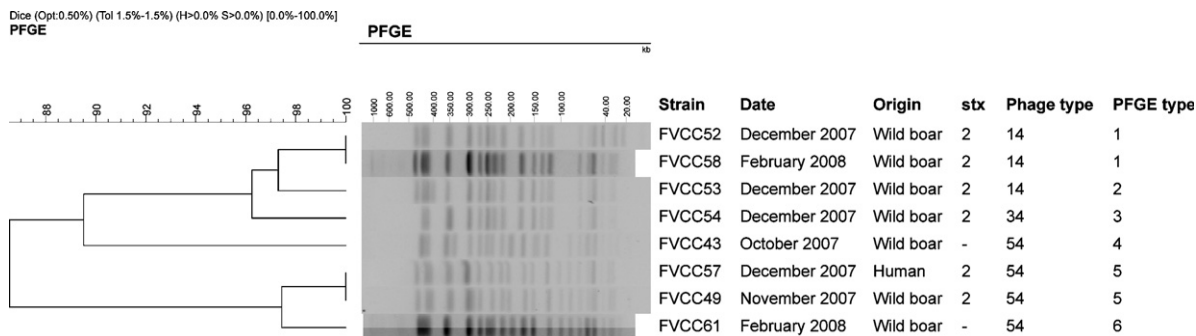
<sup>f</sup> Serotype previously found in human STEC strains.

The majority of isolates were from serotypes previously found in STEC strains associated with human infection and 8 of them belonged to serotypes (O104:H– and O157:H7) associated in particular with causing HUS (<http://www.lugo.usc.es/ecoli>; <http://www.microbionet.com.au/vtactable.htm>). The PCR procedure indicated that 4 isolates carried the *stx*<sub>1</sub> gene, 12 carried the *stx*<sub>2</sub> gene, and 1 contained both of these genes. The *ehxA*, *eae*, and *saa* genes were detected in 13, 8, and 1 of the isolates, respectively. The 8 *eae*-positive isolates comprised the types *eae*-γ1 (7 isolates) and *eae*-ζ (1 isolate), which are intimin subtypes previously reported within ovine, bovine, and human STEC strains (Rey et al., 2003; Blanco et al., 2004a,b). There were 12 different associations between serotype and virulence genes among the 19 isolates, being O157:H7 *stx*<sub>2</sub> *eae*-γ1 *ehxA* the most commonly found association (5 isolates), followed by O157:H7 *eae*-γ1 *ehxA*, O157:H21 *stx*<sub>2</sub> *ehxA*, and O23:H21 *stx*<sub>2</sub> (2 isolates each). Two isolates among the 7 *E. coli* O157:H7 isolates were *stx*-negative, although they carried genes encoding other virulence-associated factors (intimin and enterohaemolysin). Whether this finding

resulted from the loss of *stx* gene(s) from initially *stx*-positive strains remains unclear. However, whilst the human health risks associated with *stx*-negative *E. coli* O157:H7 strains may be less significant, the acquisition of *stx*-carrying phages from the environment cannot be excluded (Muniesa et al., 1999).

The *E. coli* O157:H7 isolates neither fermented sorbitol nor exhibited GUD activity after overnight incubation. Two isolates expressed the H7 antigen and five were non-motile. The seven *E. coli* O157:H7 isolates belonged to three phage types: PT14 (three isolates), PT34 (one isolate), and PT54 (three isolates) (Fig. 1). Interestingly, six of those seven *E. coli* O157:H7 isolates belonged to two of the most common phage types associated with severe human illness in Europe and Canada (Mora et al., 2007). Six distinct PFGE types (1–6) with a high degree of similarity (>86%) were found among the seven *E. coli* O157:H7 isolates recovered from wild boars (Fig. 1). The PT14 and PT34 isolates shared 96% of similarity, with two indistinguishable PFGE types and other two which differed only in ≤2 restriction fragments (Fig. 1). These PFGE types were compared with those found by us in *E. coli* O157:H7 strains isolated from human patients with diarrhoea living in the same geographic area (Martínez et al., in press). It is interesting that the PFGE type of the O157:H7 *stx*<sub>2</sub> *eae*-γ1 *ehxA* isolate belonging to PT54 recovered from the faeces of a hunter-harvested wild boar in November (PFGE type 5) was indistinguishable from that found in an O157:H7 *stx*<sub>2</sub> *eae*-γ1 *ehxA* isolate belonging to the same phage type recovered from a patient in the same area in December (Fig. 1). In the absence of epidemiologic data, these 2 isolates were also tested with multilocus variable-number tandem repeat analysis (MLVA) which fully confirmed the PFGE results (Martínez et al., 2009).

The common carriage of *E. coli* O157:H7 and non-O157 STEC indicates that hunted wild boars are a potential source of human infection, since the initial microbiological condition of the carcasses from hunted animals is generally affected by several highly variable factors and so contamination of the meat obtained from them with such pathogens would be frequent. Nevertheless, only few reports have been published on the contamination of pork from wild boar or venison with STEC (Piérard et al., 1997; Miko et al., 2009). In addition, the finding of indistinguishable PFGE types (and MLVA types) in *E. coli* O157:H7



**Fig. 1.** Dendrogram generated with InfoQuestFP software showing the PFGE-XbaI digestion types for *E. coli* O157:H7 isolates recovered from seven wild boars and a human patient. The scales at the top indicate the similarity indices (in percentages) and molecular sizes (in kilobases).

isolates recovered from samples from a wild boar and from a human patient in the same area and period suggests that wild boars in this geographic area may play a role in human infections with *E. coli* O157:H7. However, further studies will be required to further elucidate the degree of zoonotic risk posed. These findings may also have implications in the development of programs for controlling STEC and *E. coli* O157:H7 at the farm level, since wild boar populations in the studied areas have access to the same pastures as cattle and sheep, and therefore interactions between them will likely lead to interspecies transfer.

### Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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