International Journal of Food Microbiology 160 (2012) 179–182

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short communication

Detection and characterization of Shiga toxin-producing *Escherichia coli* in game meat and ready-to-eat meat products

S. Díaz-Sánchez [a,*], S. Sánchez [a,b], M. Sánchez [d], S. Herrera-León [b], I. Hanning [e,f], D. Vidal [a,c]

[a] Instituto de Investigación en Recursos Cinegéticos IREC, Ronda de Toledo s/n (CSIC-UCLM-JCCM), 13005 Ciudad Real, Spain
[b] Laboratorio de Enterobacterias, Servicio de Bacteriología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, 28220 Madrid, Spain
[c] Departamento de Ciencias Médicas. Facultad de Medicina, Edificio Polivalente, Universidad de Castilla-La Mancha, Camino de Moledores s/n, 13071 Ciudad Real, Spain
[d] EUITA-UCLM, Ronda de Calatrava s/n, 13003, Ciudad Real, Spain
[e] Department of Food Science and Technology, University of Tennessee, Knoxville, TN, USA
[f] Department of Genome Sciences and Technology, University of Tennessee, Knoxville, TN, USA

ARTICLE INFO

Article history:
Received 20 January 2012
Received in revised form 19 September 2012
Accepted 21 September 2012
Available online 2 October 2012

Keywords:
STEC
Game meat
Ready-to-eat meat products
Public health
Red deer (*Cervus elaphus*)
Wild boar (*Sus scrofa*)

ABSTRACT

A total of 142 samples of game meat and ready-to-eat meat products from red deer and wild boar were analysed in order to assess the presence of Shiga toxin-producing *Escherichia coli* (STEC). Shiga-toxin encoding genes (*stx* genes) were detected by PCR in 36 (25.4%) of the samples and STEC was isolated from 8 (5.6%) of the same samples. None of the samples tested positive for *E. coli* O157:H7. Four different serotypes were found among the 8 STEC isolates, with serotype O27:H30 being predominant (62.5%, 5/8). The PCR assay indicated the presence of the *stx2* gene in all of the STEC isolates and further subtyping resulted in detection of three different subtypes: *stx2a*, *stx2b* and *stx2g*. The only *stx1*-positive isolate was further subtyped as *stx1c*. The *ehxA* gene was detected in 3 (37.5%) of the isolates and none of them contained the *eae* gene. All STEC isolates were sensitive to the 13 antibiotics tested. Some isolates possessed serotypes and virulence gene profiles previously associated with STEC infections in humans. The isolation of a STEC strain carrying the *stx2a* subtype from a ready-to-eat meat product from deer suggests the role of these products as a potential source of STEC infections in humans.

1. Introduction

Bacterial food-borne zoonotic infections are the most common cause of human intestinal disease in many countries. For this reason, enhanced research efforts and surveillance programs are needed from government agencies with a special attention and awareness from the food industry (Newell et al., 2010). Currently Shiga toxin-producing *Escherichia coli* (STEC) are considered an important group of food-borne zoonotic pathogens causing diarrhoea, haemorrhagic colitis (HC) and the life threatening haemolytic uraemic syndrome (HUS) in humans (Hussein, 2007).

Domestic ruminants, especially cattle, are considered to be a major reservoir of STEC (Karch et al., 2005). It is also well recognised that large game animals including red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) may be healthy carriers of O157:H7 and non-O157 STEC (Díaz et al., 2011; Eggert et al., 2012; Sánchez et al., 2009, 2010). Fresh meat and ready-to-eat meat products obtained from deer have been identified as an important source of food-borne *E. coli* O157:H7 and non-STEC O157 to humans (Ahn et al., 2009; Keene et al., 1997a,b; Rabatsky-Ehr et al., 2002; Rounds et al., 2012). In spite of this, the microbiological contamination levels allowed for large game meat and meat products are not subjected to any official regulation, Furthermore, the data available regarding the microbiological quality of game meat for some pathogens is limited. A compounding issue is that the efforts on the health surveillance of wild game rely on the recognition of disease by visual inspection and recommended hygienic practices to minimise the spread and multiplication of biological hazards (Paulsen et al., 2012).

In Spain, the hunting tradition supports one of the major sources of financial income, mainly in rural areas from Castilla-La Mancha (south-central Spain) which is the main area within Spain that produces game meat (Junta de Comunidades de Castilla-La Mancha, JCCM, 2011). In this area, approximately 40,000 large game animals are shot annually resulting in revenues of 240 million of euros. Given the potential risk of large game animals as carriers of STEC and the relatively large amount of wild animals harvested in Spain, a need to determine the presence of STEC in meat and meat products obtained from large game animals in Spain exists. Thus, the aim of this study was to assess the prevalence of STEC in game meat and ready-to-eat meat products. A second aim of this work was to characterize the virulence potential of any isolated STEC. Together, this data

* Corresponding author at: Ronda Toledo s/n, 13005, Ciudad Real, Spain. Tel.: +34 926 295450; fax: +34 926 295451.
E-mail addresses: sandra.diaz@uclm.es, sandra.dsan@gmail.com (S. Díaz-Sánchez).

0168-1605/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijfoodmicro.2012.09.016

was used to determine if game meat obtained from Castilla-La Mancha, Spain may be a potential source of STEC to consumers.

2. Materials and methods

2.1. Sample collection

A total of 142 samples of game meat and game meat products were collected from three slaughterhouses and two local retail stores specialising in game meat during two hunting seasons (2009–2010 and 2010–2011) in the Castilla-La Mancha region of Spain. Samples included frozen meats (N = 84) and ready to-eat meat products, mainly fermented and dried cured meat products (N = 58) (Table 1). Each meat sample consisted of a pool of samples belonging to the same type of meat (red deer or wild boar meat originating from the same hunting estate).

2.2. Sample preparation

From each sample, a 25 g portion of meat or meat product was aseptically weighed and subsequently minced. Each sample was added to a sterile bag containing 225 ml of sterile 0.1% (w/v) buffered peptone water (Scharlab, Barcelona, Spain) and samples were homogenised for 1 min in a BagMixer® 400 lab blender (Interscience, Paris, France).

2.3. Detection and isolation of STEC

For detection and isolation of non-O157 STEC, 1 ml of the enriched culture (Scharlab) was plated onto MacConkey agar (Scharlab) and incubated at 37 °C for 18 to 24 h. Any isolates were tested for the genes encoding Stx1 and Stx2 toxins (*stx1* and *stx2* genes) by PCR as previously described (Sánchez et al., 2010). For each PCR-positive culture, up to 50 *E. coli* suspect colonies were tested for *stx1* and *stx2* genes to obtain the STEC isolates for further characterisation. If none of the assayed colonies were positive using the PCR assay, the sample was reported as PCR-positive without STEC isolation. For detection and isolation of *E. coli* O157:H7, the enriched samples were processed according to ISO 16654:2001 and presumptive colonies were confirmed as belonging to serotype O157:H7 by PCR as previously described (Sánchez et al., 2010). The resulting isolates were confirmed biochemically as *E. coli* using the API 20E system (bioMérieux, Marcy L'Étoile, France).

When STEC 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 in the same manner for further characterisation.

2.4. Characterization of STEC isolates

The serotyping of STEC isolates was conducted at the International *Escherichia* and *Klebsiella* Centre (WHO) at the Statens Serum Institut (SSI, Copenhagen, Denmark). The STEC isolates were tested for the genes encoding intimin (*eae* gene) and enterohaemolysin (*ehxA* gene), as previously described (Sánchez et al., 2010). The identification of *stx1* and *stx2* subtypes in STEC isolates was performed according to the subtyping protocol proposed by SSI. This method concerns the identification of the three *stx1* subtypes (*stx1a*, *stx1c* and *stx1d*) and the seven *stx2* subtypes (*stx2a-stx2g*) of the genes encoding Stx1 and Stx2 toxins of *E. coli* by conventional PCR amplification.

The antimicrobial susceptibility assays were conducted using the standard disk diffusion method (Jorgensen et al., 1999) on Mueller-Hinton agar plates (Scharlab) with 13 antibiotic disks (Oxoid, Madrid, Spain). Inhibition zone diameters were interpreted in accordance with the currently recommended criteria for *E. coli* (CLSI, 2011). The 13 antibiotics tested were the following: ampicillin (10 μg), amoxicillin-clavulanic acid (20/10 μg), cephalothin (30 μg), streptomycin (10 μg), kanamycin (30 μg), tetracycline (30 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), cefotaxime (30 μg), trimethoprim-sulphamethoxazole (1.25/23.75 μg), ciprofloxacin (5 μg), gentamicin (10 μg) and a compound of sulfonamides (300 μg).

Pulsed-field gel electrophoresis (PFGE) was used to establish relatedness among the STEC isolates belonging to the same serotypes. PFGE analysis with XbaI digestion was performed in accordance with the PulseNet protocol. The resulting PFGE profiles were analysed with the InfoQuestFP software version 4.5 (Bio-Rad). Isolates were allocated as different PFGE types when a single-band difference or more was detected. Cluster analysis was performed using the Dice coefficient and the unweighted pair-group method using arithmetic averages.

2.5. Statistical analysis

Differences in STEC prevalence (both in *stx* genes detection rate and STEC isolation rates) were statistically analysed by considering sample and species as variables. The statistical analysis was performed using the chi-square test for homogeneity (χ^2) (SPSS Version 19.0; SPSS Inc., Chicago, USA) and statistical significance was defined by p values ≤ 0.05, with a confidence level of 95%.

3. Results

3.1. Detection and isolation of STEC

Shiga toxin-encoding genes (*stx* genes) were detected in 36 (25.4%) of the samples, both in frozen meat and in the meat products obtained from red deer and wild boar (Table 2). Also, STEC was isolated from both frozen meat as well as meat products from red deer and wild boar (Table 2). However, none of the samples tested positive for *E. coli* O157:H7.

In the frozen meat samples, the *stx* gene detection rate (35.7%, 30/84) was higher than the meat products sampled (10.3%, 6/58) (Table 2). Also, the *stx* gene detection rate in frozen meat originating from red deer (45.8%, 22/48) was higher than samples of wild boar meat (22.2%, 8/36) (Table 2). In contrast, there were no significant differences in the *stx* gene detection rate between red deer and wild boar in meat products sampled (Table 2). A similar STEC isolation rate was observed in the types of samples, frozen meat and meat products (5.9% and 5.1%, respectively). There were no significant

Table 1
Number of samples analysed according to sample and species of animal.

	Sample origin	Wild boar	Deer	Total
Frozen meat		36	48	84
Meat products	Red cured sausages	14	9	23
	Cured sausages	14	10	24
	Dry cured meats	9	2	11
Total		73	69	142

Table 2
STEC prevalence and stx gene detection according to sample and species.

	Deer		Wild boar		Total	
	Frozen meat	Meat products	Frozen meat	Meat products	Frozen meat	Meat products
stx genes Detected	22/48 (45.8%)[b]	2/37 (5.4%)	8/36 (22.2%)[b]	4/21 (19%)	30/84 (35.7%)[a]	6/58 (10.3%)[a]
STEC isolation	4/48 (8.3%)	1/37 (2.7%)	1/36 (2.7%)	2/21 (9.5%)	5/84 (5.9%)	3/58 (5.1%)

[a] $\chi^2 = 10.366$; d.f = 1 $p < 0.001$.
[b] $\chi^2 = 4.020$; d.f = 1; $p < 0.05$.